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PREFACE

It is perhaps natural that some degree of apprehension prevails whenever a new major endeavor is undertaken. Such has been the case with the launching of the *Annual Review of Entomology*. In this instance there was no question that the job needed to be done, and our concern was limited to the best choice of methods for doing it. One estimate shows that there is appearing in world scientific literature well over 4000 papers in entomology each year. There is reason to believe that the actual number is considerably greater than this, and that it will continue to grow. Some of this literature is scattered and inaccessible, and much of it appears in journals not primarily concerned with insects. In some branches of entomology (e.g., insect biochemistry) approximately 90 per cent of the pertinent papers appear in non-entomological journals. Clearly, the mass of literature accumulating in all of the various fields of entomology is beyond the ability of any one individual to obtain readily, to read thoroughly, and to appraise critically. There has, in recent years, been a growing realization of this fact among entomologists generally. One of the solutions to the problem is to call upon specialists in the various branches of entomology to prepare for their fellow entomologists authoritative and scholarly reviews of the literature in their particular specialties.

In 1953 a committee of the Entomological Society of America was appointed to examine the problem of providing adequate reviews of entomological literature. After an exhaustive study, this committee recommended that the present needs of entomology would best be met by a review publication of the type published by the nonprofit organization, Annual Reviews, Inc., whose well-known publications already cover many fields in which the situation was similar to that now facing entomologists. This organization was approached on the matter, and, after an appraisal of its own, it also concluded that there was a real need for a review publication in the field of entomology, and it agreed to undertake the publication of an *Annual Review of Entomology*. The Entomological Society of America, in turn, agreed to assist and to give its support in a number of matters incident to the organization and publication of the *Review*.

The primary objective of the *Annual Review of Entomology* is to publish authoritative and concise treatments of definitive subjects of current interest. It is expected that the more active fields of research will require critical reviews annually while the less active areas may be summarized and evaluated as developments require. These reviews will be solicited judiciously from leaders in the fields concerned. It is our hope that each review will present a critical analysis of recent literature and, insofar as is feasible, an appraisal of the present status of the subject. We realize that such a policy may necessitate the omission of many papers that may be of

more than passing interest. Furthermore, it is not unlikely that the reviews may engender controversy because of the personal opinions and interpretations of the reviewers. However, by presenting divergent viewpoints in successive years, a well-rounded treatment of the subject as a whole should eventually be attained. It is hoped that the reviews will pursue a middle course which avoids the extremes of the personal advocacy of a restricted field on the one hand, and a mere concatenation of bibliographic summaries or abstracts on the other. The Editors and Editorial Committee invite and will always welcome suggestions for improvements.

All who read and profit from this *Review* owe a debt of thanks to Dr. J. Murray Luck and the other officers of Annual Reviews, Inc. whose unselfish interest in the needs of entomology for a review publication made it possible. Similar gratitude is due the Entomological Society of America for generously agreeing to aid in supporting the *Review* at least during its initial and formative stages. The Editors and Editorial Committee are especially grateful to Mrs. Lillian Rutherford for her skillful and invaluable aid in the editorial work. We also wish to thank the George Banta Company, Inc., for their splendid co-operation in the production of the volume. Our deepest thanks, of course, go to those who have joined in this undertaking by painstakingly preparing the reviews herein presented.

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THE PHYSIOLOGY AND BIOCHEMISTRY OF DIAPAUSE¹

By A. D. LEES

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The term diapause refers to the state of arrested growth or reproduction that is typical of many hibernating or aestivating arthropods. Although adverse conditions of various kinds may lead to the failure of growth, it is often helpful to distinguish "quiescence" attributable to such causes, from "diapause" in its more limited sense. Despite the existence of borderline examples, certain physiological mechanisms can be recognised in the diapausing insect which are absent in the quiescent. During the past decade there have been notable advances in our knowledge of these mechanisms. The role of "token" stimuli from the environment in controlling the onset and termination of diapause, the endocrinology of growth arrests, and the biochemical changes associated with dormancy are all lines of enquiry that have proved particularly fruitful. The present article is concerned especially with these topics. General reviews in which the ecological and phenological, as well as the physiological aspects of diapause are discussed, include those of Bonnemaïson (7), Andrewartha (2), and Lees (62).

THE INDUCTION OF DIAPAUSE

Photoperiod.—Recent investigations have shown that some form of response to length of day is extremely common among arthropods possessing a facultative diapause. In the majority of species long day lengths or permanent illumination prevent diapause while 8 or 12 hr. days induce diapause. Very short photoperiods or permanent darkness often cause uninterrupted growth. This general pattern of response has now been observed in the Lepidoptera *Acronycta* (18), *Grapholitha* (25), *Cydia* (25), *Polychrosis* (53), *Diatraxia* (101), *Antheraea* (94, 95), *Pieris* (21), *Harrisina* (92), and *Araschnia* (18, 67), as well as in the Colorado beetle *Leptinotarsa* (103) and the red mite *Metatetranychus* (60). The photoperiodic response is also of this "long day" type in certain Lepidoptera which hibernates as larvae. But there are important differences. In *Dendrolimus* (Lasiocampidae) larval feeding and moulting are soon arrested in short days, irrespective of the instar. But, unlike the species mentioned above, *Dendrolimus* remains susceptible to day length throughout the period of hibernation, so that growth is resumed a week or two after exposing the larvae to a long photoperiod (37, 38). Gayspitz has also shown that the larvae of *Euproctis chrysorrhoea* (Linnaeus) and other lymantriid moths are sensitive to photoperiod, notwithstanding their strict univoltine rhythm in nature. But diapause is

¹ The survey of the literature pertaining to this review was completed in May, 1955.

only prevented by photoperiods corresponding to extreme long-day conditions (17 to 22 hr. days in the case of a Leningrad population). In this instance the absence of light, and even permanent illumination, causes the larvae to become dormant. Postdiapause growth is also facilitated by an optimal photoperiod of about 20 hr., but it is less certain whether the larvae are still photosensitive when fully dormant. The silkworm *Bombyx* is exceptional in that the direction of the response is reversed, short day lengths preventing, and long day lengths inducing diapause (49). This reversal is associated with the fact that the eggs are influenced by illumination almost immediately after development is resumed in spring. Under natural light conditions, therefore, the first generation moths of bivoltine strains lay non-diapause eggs in summer and the second generation diapause eggs in early autumn.

The mode of action of light has been examined in several species. *Grapholitha* and *Metatetranychus* are known to take account of day length as such (25, 60). But there is some evidence that the dragonfly *Anax* responds to changes in day length. Increasing photoperiods cause the last stage nymphs to metamorphose promptly, whereas decreasing or constant long photoperiods induce diapause (16). The light reaction itself seems always to be independent of intensity, provided the latter is not subliminal. The threshold is as low as 0.01 ft-c in the eggs of *Bombyx* (49) and about 1 to 2 ft-c in the deutonymphs of *Metatetranychus* (60). *Grapholitha* responds to a light intensity of 1 to 3 ft-c falling on the outside of the apples in which the larvae are tunneling (25). Although accurate action spectra are not yet available, the blue and blue-green regions of the spectrum are known to be especially effective in *Bombyx*, *Grapholitha*, and *Metatetranychus*, all three species being virtually insensitive to red light. The nature of the photosensitive pigment is still obscure.

Both the light and dark phases of the cycle of illumination are involved in the control of diapause. The role of the dark phase has been demonstrated in *Acronycta* by Danilyevsky & Glinyanaya (22, 23) who have found that diapause pupae are only formed if the dark period is longer than 9 hr. and shorter than 48 hr. In *Antheraea* the pupae are always of the diapause type if the dark phase exceeds 11 hr., apparently regardless of the duration of the light period (95, 96). However, the mode of action of the rhythm of illumination has been more closely studied in *Grapholitha* (25) and *Metatetranychus* (61). In the first species the conditions which evoke diapause are extremely limited: the duration of the light phase must fall between 7 and 15 hr. and that of the dark phase between 11 and 16 hr. All cycles in which either component is longer or shorter than these values cause the larvae to pupate without diapause. The conditions which lead to the production of females laying diapause winter eggs are much less restricted in *Metatetranychus*. The type of egg laid appears to be determined by the interaction of antagonistic diapause-preventing (light phase) and diapause-inducing (dark phase) proc-

esses. The light and dark phases both become more effective with time, the latter showing a particularly dramatic gain in effectiveness between 8 and 12 hr. Nevertheless, both reactions are slow to start, and for this reason diapause is not influenced by the inclusion of short light breaks within an effective dark period. This is also true of *Grapholitha*. Indeed, in this respect, as in others, the photoperiodic reactions of arthropods seem to differ materially from those in plants. As a first approximation, it is tempting to conclude that some active product is synthesised in the light or dark and removed during the complementary phase. Since the number of effective cycles that fall within the sensitive period of development exert a cumulative effect (60), there must also be some means of transferring the substance from one cycle to the next.

It has been possible to show in *Diataraxia* and *Metatetranychus* that diapause is not governed by photoperiodic reactions set up in the food plant (60, 101). Yet although the action of light is evidently direct, the site of photoreception has still to be identified. Since Tanaka (95) has found that the lateral ocelli are not the receptors in the larvae of *Antheraea*, it may well be that light affects an internal organ, perhaps the central nervous system itself. Some indirect information on this question is provided by studies of the sensitive period.

Photoperiod often operates on the insect long before growth is finally arrested. The timing of the sensitive period varies considerably according to species. In *Bombyx* it precedes the stage of arrest by almost an entire generation, the type of egg being decided by the conditions experienced by the moth during late embryonic development (49). In *Polychrosis* the pupal diapause is also largely determined in the egg stage (53), while in *Araschnia* the fate of the pupae is not decided until the fourth and fifth larval instars (18). *Leptinotarsa* will still respond to a long photoperiod up to the time when it is entering the soil to hibernate (103). And red mites laying summer or winter eggs can be induced to switch over to eggs of the opposing type by exposure to an antagonistic photoperiod (60). The duration of the sensitive period is also a variable feature. In *Diataraxia* it occupies no more than two days during the "moulting sleep" of the penultimate instar (101); in contrast, all the larval instars of *Antheraea* show some sensitivity although the last two are probably more critical, as can be demonstrated by rearing the other instars in a "neutral" or intermediate day length (93, 94).

Since the interval between the sensitive period and the stage of arrest does not bridge the early stages of embryogenesis in any of these species, it need not be assumed that the effect of photoperiod is transmitted through the cytoplasm of the egg. This is true even of *Bombyx* where diapause is essentially a maternal character (p. 8). These results are therefore compatible with the view that some organ system, such as the central nervous system, is concerned in transmitting the stimulus. Nevertheless, it should be noted that in the pteromalid *Spalangia* and the blowfly *Lucilia*, there is

some evidence that the larval progeny is influenced by physical factors acting on the maternal generation (17, 90).

Temperature.—Diapause is seldom independent of temperature except in species like *Antheraea* (93) and *Harrisina* (92) where photoperiod has assumed a dominant role. As a rule, diapause is suppressed by high temperatures and induced by low. This response has been recorded in *Diataraxia* (101), *Chilo* (29), *Orgyia gonostigma* Linnaeus (54), *Eurydema* (8), *Metatetranychus* (60), and in many other arthropods. It is interesting that in *Bombyx* high temperatures and long photoperiods also act in unison, but the direction of the response is reversed (49).

Food.—There is little evidence that diapause is often brought on by changes in the diet. The low water and high fat content of the ripening cotton boll may be of some significance in *Platyedra* (28). The onset of diapause in *Euproctis chrysorrhoea* (Linnaeus) has been attributed to the age of the foliage (41). But this now appears doubtful as more recent researches have shown that day length, and not the larval food, is the dominant factor in this species (38). Nevertheless, diet exercises a marked effect on *Metatetranychus*. When the food consists of yellowing, senescent leaves or "bronzed" foliage (i.e., leaves damaged by the feeding punctures of other mites) winter eggs are laid, even though the conditions of temperature and photoperiod are such as to prevent diapause strongly. The effect may be one of simple starvation (60).

THE TERMINATION OF DIAPAUSE

It has long been recognised that the release from diapause is often favoured by a long exposure to temperatures that are too low to permit morphogenesis. When the rates of completion of diapause are compared with rates of growth, certain points of resemblance emerge: both processes take place only within a certain temperature range and proceed most rapidly at a well-defined temperature optimum. These considerations have led Andrewartha (2) to coin the term "diapause development" to describe the gradual process of physiological development which he believes must take place as a prerequisite to the resumption of growth. This concept is clearly of considerable utility even if it should eventually transpire that the mechanism involves nothing more than the synthesis of a comparatively simple series of chemical substances.

The temperature requirements for diapause development differ to a remarkable extent. The connection with geographical distribution has recently been emphasised by Danilevsky (19). In most saturniid moths, for example, the temperature range compatible with morphogenesis is roughly the same (ca. 15 to 32°C.). But in the Palearctic *Saturnia pavonia* Linnaeus the range which is favourable for diapause development extends from 7°C. down to almost -15°C., so that this species can never develop in constant temperature conditions. Diapause is completed at rather higher tem-

peratures in *Antheraea pernyi* Guérin (112), and in the subtropical species *Philosamia cynthia* (Drury) the ranges for diapause development and growth overlap broadly but are still by no means coincident. It is now clear that the temperature optimum is often relatively low in species from cold or temperate climates. The optimum is 7°C. in *Bombyx* (27, 68, 114), 10°C. in *Cephus* (78), *Epistrophe* (82), and *Aporia* (113). And approximately the same temperatures are effective in *Melanoplus bivittatus* (Say) (15) and many European Lepidoptera (19, 55). Species from warmer environments require correspondingly higher temperatures. The optimum in the Australian cricket *Gryllulus* is 13°C. (10), in the beetle *Anthrenus verbasci* (Linnaeus) nearly 20°C. (57). Other species with high optima include *Locustana* (66), *Leptinotarsa* (103), *Harrisina* (92), and the subtropical silkmoth *Eriogyna* (52). These requirements are usually satisfied during the winter or cool season, although, if the winters are severe, autumn and spring may be more favourable for diapause development (112). However, when the temperature optimum is relatively high, and diapause of no great intensity, the insect may be released from dormancy in autumn instead of the following spring. This aberrant type of life cycle is found in the winter moth *Operophtera* (56).

The duration of diapause is also variable. It is quite transient in the pentatomid *Eurydema*, disappearing after only 9 days at a temperature of 8°C. (8). In *Bombyx* 60 days at 5°C. suffice (68), while *Metatetranychus* requires 150 to 200 days (60). Many Lepidoptera with an intense diapause may lie dormant for several years (20). And the gall midge *Sitodiplosis* sometimes passes up to 12 winters in the soil (3). Another interesting feature is that the responsiveness to chilling often depends on the stage of morphological or physiological development. This is particularly evident in insect eggs. Thus the *Gryllulus* egg is most sensitive some days before the embryo has actually developed to the diapause stage (9). In contrast, diapause disappears most rapidly in the *Locusta* egg if the period of chilling comes after about 60 days of high temperature (that is, 50 days after embryonic development has ceased) (59). These observations show that the temperature optimum sometimes changes during the course of "diapause development." Nevertheless, very little is known about the mechanism itself. Since one would not expect that any chemical reactions catalyzed by enzymes would possess a negative temperature coefficient, it is necessary to assume that "diapause development" involves the interaction of two or more processes, perhaps competing for the same substrate.

The role of water.—Many orthopteran eggs, as well as hibernating larvae and adult insects, cannot begin their postdiapause growth until moisture has been taken up from their surroundings. When water is lacking, diapause is often prolonged by a period of quiescence. In such cases it is often difficult to decide whether the processes controlling water uptake form an integral part of the diapause mechanism. Nevertheless, if this were so, one might expect that active uptake would remain in abeyance until diapause develop-

ment was completed. This appears to hold good in *Locustana*, for eggs of the diapause type will replace any water lost by evaporation, thereby restoring their turgor; but they will not take up sufficient water to stretch the membranes until diapause has disappeared (66). In *Melanoplus differentialis* (Thomas) the entry of water is cut off at the beginning of the diapause period by the deposition of a waxy layer over the hypoderm, so that diapause can be artificially ruptured by immersing the egg briefly in xylol (91). Indeed Bucklin (12) has shown that dormant embryos, when removed from their membranes, will even develop in Ringer's solution. It is therefore reasonable to assume that the limiting factor in these eggs is water lack and that the resumption of growth is dependent on the active water-absorbing properties of the extra-embryonic tissues. The eggs of *Dociostaurus* possibly provide a further example of this type of relationship (4). Nevertheless, a similar interpretation cannot always be applied. If the freshly laid eggs of *Gryllulus* are incubated in moist conditions, almost all the water is imbibed in the first few days so that the eggs enter diapause in a fully hydrated condition (11). Water absorption is also virtually complete when the almost fully developed embryos of *M. bivittatus* become dormant (79, 80).

Similar differences are found among larval and adult insects. In the borer *Chilo* no water at all is taken in until diapause development has been completed (50, 51). Yet diapausing larvae of the sawfly *Cephus* absorb water whenever it is available. In the usual way uptake proceeds steadily during the 100 or so days of chilling that are needed to end diapause. But if the larvae are chilled without access to water the entire amount can be taken up during the postdiapause period. Diapause development in *Cephus* is not retarded by dehydration (13). Contact water is also necessary for prompt development in the gall midges *Mayetiola* (111), *Schizomyia* (69), and *Contarinia* (40), as well as in the weevil *Rhynchites* (43). However, diapause mechanisms seem to be lacking except perhaps in the first species. The physiology of these types of arrest has not yet been studied in detail, but it seems probable that dehydration inhibits the secretory activity of some component of the endocrine system, perhaps the prothoracic glands in the case of larvae.

THE INHERITANCE OF DIAPAUSE

Strains differing in the character of their diapause have been recognised in many arthropods. The inherited differences always appear to involve the response to the many agencies which elicit or terminate diapause. For example, geographical strains of *Tetranychus* respond to slightly different critical photoperiods (6, 60, 62); the northern Japanese race of *Chilo simplex* Butler has a more intense diapause than the southern (110); the West Australian race of *Austroicetes* has a rather higher temperature optimum for diapause development than the South Australian race (2). A more far-reaching adjustment to the climatic conditions prevailing within the area of dis-

tribution is seen in species, such as *Pieris napi* (Linnaeus), which are represented by races with facultative and obligatory diapause. Peterson (74) has found that obligatory diapause, and a univoltine life cycle, predominates in northern Scandinavia, whereas genotypes conferring facultative diapause become increasingly frequent towards the south as the favourable season becomes longer. The same is probably true of populations of *Pyrausta* in the Lake States (U.S.A.) (70, 109). Subtropical populations of *Locusta migratoria* Linnaeus show no form of arrest, but an egg diapause has appeared in the subspecies *gallica* from the Bordeaux region and in other isolated populations near the limits of distribution (58, 59). Nevertheless, diapause races are sometimes associated with ecological rather than geographical isolation. For example, in the mite *Bryobia* strains without diapause and others with facultative and obligatory diapause have developed in association with different host plants (65).

The genetics of diapause has been examined only in *Bombyx* (98). Several genes, both sex-linked and autosomal, affect the voltinism. They have been assigned different "hibernating values" which are recognised by the influence of temperature on the induction of diapause. By suitable recombination experiments it is possible to prepare a series of stocks showing a graded response to temperature. These range from multivoltines (no apparent diapause), through bivoltines to univoltines (virtually obligatory diapause). As all the intermediate grades are represented, it is quite clear that the genotype modifies and shifts the response of the "receptor mechanism" to environmental factors such as temperature (62). There is no doubt also that the extreme types should be regarded as responding to the environment; in the case of an obligatory arrest it is merely that the range of stimuli that are diapause-inducing has become so broad that the insect can rarely escape this condition. Selection experiments with species such as *Ephestia* (100), *Locusta* (59), and *Antheraea* (97) have also thrown light on this question.

DIAPAUSE IN PARASITIC INSECTS

The synchronous development of insects and their parasites is achieved in different ways. One type of relationship has recently been studied by Schneider (83, 84). The eggs of *Diplazon fissorius* Grav. (Ichneumonidae) are deposited in young syrphid larvae, but although they soon hatch, the first instar larvae undergo no further development until the host forms the puparium. In the multivoltine syrphid, *Epistrophe balleata* Deg. the delay lasts only a few days, but in the univoltine species *E. bifasciata* Fabricius which has a nine-months larval diapause, the parasite also overwinters as a young larva. It is certain that the limiting factor is not the actual development of the host as the parasite is unaffected by the larval moults. It is rather some event (presumably of a chemical or humoral nature) which is peculiar to pupation. As the parasite larvae are very rapidly activated or immobilised

when transplanted into the appropriate hosts, this state of arrest resembles "quiescence" rather than diapause.

Many parasites exhibit a second form of arrest, a definitive diapause which supervenes at the close of the last larval instar after the host has been consumed. The assumption has often been made that some "diapause substance" is ingested and that this in turn inhibits the growth of the parasite. However, recent work by Gayaspitz & Kyao (39) suggests that the braconid *Apanteles glomeratus* (Linnaeus) responds independently to length of day when developing in *Pieris brassicae* (Linnaeus). Both parasite and host are "long-day" insects, but, in order to suppress diapause, *Apanteles* requires slightly longer photoperiods than *Pieris*. When reared at this critical photoperiod, therefore, the parasites escape diapause although most of the hosts would enter diapause if they survived. A more direct proof is furnished by the fact that dormancy can still be prevented by exposing the larvae of *Apanteles* to long days immediately after they have left the host, even though the parasitized *Pieris* larvae have previously received short-day treatment. Day length is also involved in releasing the scelionid egg-parasite *Telenomus* from diapause.

It is well known that certain Diptera which normally overwinter as diapausing third instar larvae, form their puparia prematurely in autumn when invaded by hymenopterous parasites. Implantation experiments with the ichneumonid *Diplazon pectoratorius* (Thunb.) have revealed that the parasite does not activate the host's endocrine centres; nor is the wound puncture of any significance. Induced pupation is a result of the direct action of some substance secreted by the parasite. This may either be saliva or, more probably, the moulting hormone which diffuses out through the body wall of the parasite (84).

THE HORMONAL REGULATION OF DIAPAUSE

It now appears probable that humoral controlling mechanisms are implicated in all forms of diapause, whether embryonic, larval, pupal, or imaginal; but the endocrine systems differ according to the stage of arrest. The long series of investigations by Fukuda (30 to 36) and Hasegawa (44) on *Bombyx* has shown that the type of egg is decided by a secretion liberated from the suboesophageal ganglion. In the presence of this hormone, which is presumably derived from neurosecretory cells, diapause egg batches are laid; in its absence nondiapause batches are laid. Although the voltinism is normally determined by the environment acting on the late embryo, the hormone is not secreted until the pupal stage. A pupa initially determined for the production of nondiapause eggs will, therefore, develop into a moth laying diapause eggs if a suboesophageal ganglion is implanted. The suboesophageal ganglion is itself controlled by the brain through the circumoesophageal commissures. In the insect determined as a nondiapause egg-producer the brain seems to inhibit the secretion of the hormone. This restraint disappears

when the connectives are cut. It follows that suboesophageal ganglia from either diapause or nondiapause donors are capable of converting the eggs in a "nondiapause" recipient to the diapause condition. It is less certain, however, whether the brain merely ceases to inhibit the ganglion in the diapause egg-producer or whether it actually stimulates the synthesis or release of the active product. Suboesophageal ganglia from insects belonging to different races all have the same potentialities when isolated. The difference arises from the behaviour of the brain: in multivoltine insects the brain inhibits, and in univoltine insects activates, the suboesophageal ganglion without apparent regard to the previous environmental conditions (see, however, p. 7). A similar hormone is released by the suboesophageal ganglion of the male *Bombyx*, and even by those of certain species (e.g., *Antheraea pernyi*) with a pupal diapause.

Despite this clear evidence of regulation by the maternal endocrine system, the more immediate reason for the growth failure in the *Bombyx* egg remains uncertain. In *Melanoplus differentialis* the cause of the arrest does not reside within the yolk or even within the embryo itself, for mitotic activity is immediately resumed when diapausing half-embryos or even isolated limb rudiments are cultured *in vitro* (12). In this egg the growth failure seems to be attributable to a physical factor, namely an insufficiency of water (p. 5). Possible ways of reconciling this apparent contradiction are discussed later (p. 12).

It is well known that moulting and growth are controlled by an endocrine system which includes the brain and prothoracic glands. Researches by Williams (104 to 107) have shown that the pupal diapause in *Platysamia* is the result of the failure by the neurosecretory cells of the brain to secrete the hormone which is required for the activation of the prothoracic glands. There is no reason to attribute the growth arrest to the presence of an inhibitory principle, for when an intact diapausing pupa is joined in parabiosis with a developing pupa both members of the pair develop promptly. These conclusions have been confirmed in *Actias selene* Hübner (107) and *Luehdorfia* (47), as well as in certain insects with a diapause in the larval stage such as in *Gryllus* (88) and *Cephus* (14). And cytological evidence suggests that neurosecretion is lacking during the dormant period of the sawfly *Lophyrus* (45). Nevertheless, the brain is not invariably responsible for the interruption in the cycle of endocrine activity. In *Sialis* and the swallowtail *Papilio xuthus* Linnaeus the prothoracic glands apparently fail to activate the tissues (71, 77). The evidence is considered below.

The action of temperature on this endocrine system is particularly important in the present context. Like many other insects, *Platysamia* requires an adequate exposure to low temperature before development can be resumed. There is little doubt that the site of action is the brain itself. Thus unchilled brains that are transplanted into brainless diapausing pupae acquire the ability to secrete the neurohormone after the "host" pupae have

been chilled; at the same time unchilled brains do not become competent after transplantation into chilled brainless pupae [Williams (personal communication)]. This must mean that the normal afferent nerve supply to the brain is not an essential part of the mechanism; nor is the brain activated by diffusible substances liberated by other chilled tissues. The action of temperature on the brain is not fully understood, but it is helpful to distinguish between the gradual development of competence to secrete the neurohormone (this is essentially the process of "diapause development") and the subsequent release of the active principle into the haemolymph: the first process often requires a low temperature, the second a much higher temperature (108).

The organs responsive to chilling in *Sialis* and *Papilio* seem to be the prothoracic glands. Chilled larvae of *Sialis* develop promptly even after brain removal. Apparently the prothoracic glands require a stimulus from the brain, just as in other insects, but this takes place before the larvae have entered diapause (77). Decapitated diapausing pupae of *Papilio* will not develop at high temperatures, but they respond in the normal way to chilling (71). An interesting temperature effect, which has been noted in *Cephus*, relates to the re-establishment of diapause when adequately chilled larvae are exposed for short periods to a high temperature of 35° or 40°C. It seems that this treatment halts the secretion or release of the prothoracic gland hormone and thereby permits the brain neurohormone to be dissipated (14).

Reproductive diapause in *Dytiscus* (48), *Leptinotarsa* (103), and *Anopheles* (24) is known to be associated with the absence of the gonadotrophic hormones that are necessary for the full maturation of the ovaries. Although the hormone secreted by the corpus allatum is the immediate agent, a more remote control is probably exercised by the brain in the case of *Dytiscus* (48) and *Leptinotarsa* (42).

METABOLISM AND DIAPAUSE

Oxygen consumption during hibernation often falls to little more than one-tenth of the value in the growing or reproducing insect. These trends in the intensity of respiration have recently been followed in the *Platysamia* pupa (85) and in the tenebrionid beetles *Anatolica* and *Opatrum* (26). The level of this "maintenance metabolism" is probably lowest in species with an intense diapause: this results in the slow utilization of the food reserves and lengthy survival (20). The initial pathways of oxidative metabolism during growth and diapause may well be alike. It is true that extremely low respiratory quotients have sometimes been recorded in dormant insects; and this has led to the suggestion that fat is converted into carbohydrate (1). Nevertheless, it is now known that many hibernating larvae and pupae conserve their water supplies by opening the spiracles intermittently. Carbon dioxide is therefore accumulated and only released in "bursts" every few hours (76). After allowance had been made for this circumstance a respira-

tory quotient of 0.78 was obtained for *Platysamia*, a value consistent with the utilization of a mixture of carbohydrate and fat (85).

Attention has been centered more particularly on the biochemical adjustments which are responsible for these quantitative changes in respiration. It has been claimed on very slender evidence that the pupal diapause in *Phalera* is caused by a vitamin deficiency which interferes with the process of decarboxylation (1). On the other hand, the important contributions of Williams and his co-workers have shown that diapause in *Platysamia* is associated with significant changes in the terminal oxidase systems. The cytochrome pigments, a group of conjugated proteins with a prosthetic group allied to haem, occupy a central position in this connection. The diapausing pupa is almost completely resistant to inhibitors of cytochrome oxidase such as carbon monoxide and cyanide. And respiration is scarcely affected by them (86, 87). The pupae also survive the injection of pilocarpine and diphtheria toxin, agents which are believed to block the synthesis of certain components of the cytochrome system. In contrast, pupae that have begun to undergo metamorphosis, as well as adult insects, are readily killed by these respiratory poisons (72).

Spectroscopic studies have shown that in addition to the classical cytochromes, *b*, *c* and *a*+*a*₃, a further component, *b*₅ (*b*_x or *e*) is especially important and probably represents the terminal oxidase in the diapausing pupa (81, 89). The properties of this haemoprotein have been examined by Pappenheimer & Williams (73). Like other members of the *b* group it is auto-oxidisable and therefore unaffected by inhibitors of cytochrome oxidase. In larval mid-gut preparations (which are rich in this enzyme) a considerable part of the total electron transfer passes directly from coenzyme I (DPNH)² to cytochrome-*b*₅ and thence to molecular oxygen. This cytochrome does not participate to any appreciable extent in the oxidation of succinate; indeed, because of the lack of cytochrome-*c* in these preparations, the addition of succinate completely fails to stimulate respiration.

Cytochrome-*b*₅ is present in all the tissues of the diapausing pupa, together with *a*+*a*₃, but cytochrome-*c* is entirely absent except in the inter-segmental muscles of the abdomen. These elements, therefore, degenerate when the pupa is treated with cyanide. After the resumption of development cytochrome-*c* rapidly appears in the tissues while cytochrome-*b*₅ is reduced to the merest trace. Williams has pointed out that the low diapause metabolism of the pupa is particularly associated with the presence of cytochrome-*b*₅ and with the virtual absence of *c*, a system which appears to be incompatible with growth. When diapause is ended, the continuation of growth, and even at this stage the maintenance of life, is dependent upon the rapid synthesis of cytochrome-*c* and the development of a metabolism catalyzed by cytochrome oxidase.

² diphosphopyridine nucleotide (reduced form).

It is well known that the respiration of the diapausing *Melanoplus* egg is also of the CO- and cyanide-stable type. But the addition of succinate to egg homogenates greatly stimulates respiration; and this induced respiration is inhibited by cyanide. The development of a cyanide-sensitive metabolism following the resumption of embryonic growth has, therefore, been attributed to the "recoupling" of a succinoxidase system which is intact but inactive in the diapausing embryo, rather than to the synthesis of cytochrome-*c* (5). In other species such as *Leptinotarsa* (75) and *Pristophora* (64) cyanide-sensitivity is retained throughout dormancy. And in *Popillia* cytochrome oxidase activity is particularly high at this time (63). Presumably the respiratory changes in these insects must involve some other link in the long chain of oxidative processes.

CAUSAL MECHANISMS IN DIAPAUSE

While the biochemical adjustments discussed in the preceding pages are clearly a most essential part of the diapause mechanism, they are not themselves causal, since they are evoked by physical or humoral changes taking place outside the tissues. In *Platysamia*, for example, slight wounds cause a dramatic rise in oxygen consumption; yet although wound-healing involves the temporary restitution of a metabolism mediated by cytochrome oxidase, growth is not resumed. The reason is that the hormone from the prothoracic glands is still lacking (85). Nevertheless, despite general agreement that hormones play a central part in the control of diapause, their precise role is still disputed. Some writers attribute embryonic arrests to the presence of a "diapause hormone" with an inhibitory action; and this hypothesis has even been extended to include larval and pupal diapause (46). The available evidence hardly favours this view. Present indications are that larval and pupal arrests are attributable to a hormone failure, often the neurohormone from the brain, but sometimes apparently the secretion from the prothoracic glands.

On the other hand, we have seen that the diapause condition of the *Bombyx* egg is determined by the presence of a hormone in the maternal haemolymph (p. 00). It is frequently assumed that the secretion from the suboesophageal ganglion or some other "voltinism determiner" or "hibernation substance" persists within the egg after diapause has set in and is only gradually dissipated (49, 68). However, it is perhaps equally probable that the relationship is indirect: the immediate cause of the growth failure may be the existence of unfavourable physical conditions within the egg. The limiting factor may well vary in different eggs but water lack (12, 91, 99) and an oxygen deficiency are two possibilities. If the exchanges with the external environment are controlled by the extra-embryonic membranes, the function of the hormone from the suboesophageal ganglion may be to elicit these powers of regulation.

Another interesting question concerns the perception of such environ-

mental agencies as photoperiod and temperature. Present evidence, although meagre, suggests that their action on the brain may be more or less direct. But it is still entirely obscure how they determine the "programme" of neurosecretion, often many instars ahead of the actual arrest of growth. The same problem is of course encountered in the study of metamorphosis, since a comparable mechanism for "counting the instars" must be invoked to explain the constant number of larval moults in many species (102). It is interesting to note that this function must also be assumed to reside in the central nervous system.

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INSECT NUTRITION^{1,2}

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With some exceptions, this survey is restricted to material published since the extensive reviews of Trager (253) and Wigglesworth (265). A working knowledge of these contributions as well as of the fundamentals of nutrition is assumed on the part of the reader [see Bourne & Kidder (27)]. In some instances only the latest or the most complete of a given author's investigations into a specific problem will be cited in order to conserve space for critical discussion.

Recent advances in the study of feeding mechanisms, disease transmission, and the nutrition of predators and soil or wood inhabiting insects will not be discussed because of limitations of space or the absence of significant new data. The methodology of insect culture is exhaustively treated by Peterson (210), including a review of methods available for the labelling of insects by the feeding of radioactive materials [also see Hinton (115)]. Other compendiums of interest include a treatment of comparative aspects of the nutrition of the various phyla by Vonk (261) and of the food requirements of Crustacea [Beerstecher (17)].

THE REQUIREMENTS OF INSECTS FOR SPECIFIC NUTRIENTS

Albritton (1) has edited a summary of the food requirements of microorganisms, plants, and animals, in which the data are presented in tabular form. The sections devoted to insects present exhaustive tables dealing with the formulation of synthetic diets and the utilization of proteins, amino acids, carbohydrates, lipides, and essential organic and inorganic dietary elements. The increased use of purified diets has focused attention on the factors which contribute to anomalous findings. In the case of dry diets the necessity for proper particle size is stressed by Fröbrich (89). The sexing of populations may be necessary with species where the male and female differ significantly in size, as in the roach [Hilchey (112), Hilchey & Patton (114)]. The need for genetically homogeneous populations of roaches has been indicated by Noland *et al.* (199), as has Leclercq for *Tenebrio molitor* (166), and Magis for various *Tribolium* species (183, 184).

The inclusion of small quantities of yeast in synthetic diets for insects has been a general practice. Whether this supplement is effective for reasons of increased palatability or through the provision of missing nutrients may

¹ The survey of the literature pertaining to this review was completed in June, 1955. The authors have omitted numerous contributions which, but for restrictions of space, should rightfully be included in this review.

² The following abbreviations are used in this chapter: CoA for coenzyme A; DNA for deoxyribonucleic acid; 3-OHAA for 3-OH-anthranilic acid; 3-OHK for 3-OH-kynurenine; RNA for ribonucleic acid.

vary from species to species, but King (144) has shown that the inclusion of yeast in the diet of *Drosophila* favors phosphorus uptake far beyond the degree indicated by the phosphorous content of the yeast. The feeding of Cu^{64} to *Drosophila* larvae has demonstrated different routes of absorption for free ionic copper and for tissue-bound copper presented in the form of yeast reared on Cu^{64} media [Paulson *et al.* (208)]. The effect of uncharacterized substances present in various foods may be sufficiently marked as to seriously question the use of the term "synthetic" for rations containing yeast, nucleic acids, and even such seemingly innocuous materials as starch and fatty oils. Significant nutritional differences for insects between brands of "vitamin-free" casein have been shown by Fraenkel & Blewett (75) and Fröbrich (90).

Amino acid and protein requirements.—The present state of our knowledge of protein and amino acid requirements is well summarized in the tables gathered by Albritton (1). Results obtained with six different species of insects indicate that these forms require the same 10 amino acids classified as essential for the rat.³ The larvae of *Drosophila melanogaster* have been reared upon a synthetic medium by Hinton *et al.* (117, 118). Under aseptic conditions, good growth was obtained with the 10 essential amino acids, glycine, and seven nonessential amino acids, including serine. Goldsmith (98) has reported on the ability of azaserine to act as an antimetabolite for serine in *Drosophila*. The effect is reversible by feeding the natural amino acid. The parasitic dipteran larva *Pseudosarcophaga affinis*, has been shown to require the 10 essential amino acids when reared aseptically and to benefit from the presence of the nonessentials alanine, glycine, serine, and tyrosine [House (123)]. Ribonucleic acid (RNA) was a necessary constituent of this regimen. Nineteen amino acids have been shown to satisfy the protein requirements of the onion maggot, *Hylemya antiqua* [Friend & Patton (87)], while glycine plus the 10 essentials will support the blowfly, *Calliphora erythrocephala* [Sedee (227)]. The blowfly and the onion maggot also require a source of nucleic acid when grown axenially. Weight gain by the imago of the honey bee worker representing mainly the growth of the pharyngeal glands, requires the 10 essential amino acids and is stimulated by the inclusion of glycine, urea, or ammonium salts in the diet [DeGroot (50)]. The same 10 essential amino acids were shown to suffice for the development of *Tribolium confusum* by Lemonde & Bernard (172) and later by Fröbrich & Offhaus (91). These rations contained small amounts of yeast as well as starch and were not sterile.

T. confusum was successfully grown in the absence of yeast by Fraenkel & Printy (79). Although the 10 essentials supported growth of this species, the addition of nine nonessential amino acids resulted in a stimulation of the rate of weight-gain.⁴ Feeding of the D (unnatural) form of the 10 essential

³ Arginine, lysine, leucine, isoleucine, tryptophan, histidine, phenylalanine, methionine, valine, and threonine.

⁴ Unpublished work of the senior author (H.L.) has shown that the firebrat, *Thermobia domestica*, may be reared on the diet devised by Fraenkel & Printy (79).

acids showed D-methionine, D-phenylalanine, and possibly D-lysine to be utilized by the larvae. The other seven D acids were inactive, but not toxic. *T. molitor* and *D. vulpinus* did not develop on amino acid diets. Leclercq (165) by the use of zein diets has shown that *T. molitor* requires lysine and tryptophan.

The ingenious studies of House (122) concerning the amino acid requirements of the German roach have been extended by Hilchey (112), who added alanine, lysine, and the leucines to the list previously shown to include valine, tryptophan, histidine, arginine, and serine in the case of the male. The female could dispense with serine. Apparently *Blatella germanica* can synthesize the phenyl moiety of the aromatic amino acids, phenylalanine and tyrosine, or perhaps derive it from tryptophan. This species can also convert inorganic sulfur to protein sulfur and the $-S-CH_3$ group [Hilchey *et al.* (113)]. Noland & Baumann (198) had previously reported on the utilization of different proteins by *B. germanica*. These ranged from good for casein and fibrin, to fair for steamed egg albumin, lactalbumin, and wheat gluten, and poor for enzymatically hydrolyzed casein, zein, oxidized casein, gelatin, and hemoglobin. Roaches reared to maturity on oxidized casein, which is deficient in tryptophan and methionine, nevertheless accumulated normal quantities of both amino acids in their tissues. Growth in the absence of dietary methionine could be suppressed by feeding of 1 per cent sulfasuxidine. Growth of the cockroach in the absence of certain amino acids and on proteins of poor quality can be explained by the presence of intracellular symbionts which do not respond to the ordinary bacteriological tests for sterility. The taxonomy of some of these microorganisms has been treated by Vollbrechthausen (260), and the ability of these forms to synthesize growth factors has been reported by Brooks & Richards (30). It has been shown by Haydak (107) that an optimum dietary level of protein (casein) exists for the roaches *P. americana*, *B. germanica*, and *B. orientalis* beyond which life is foreshortened. It was further reported that high levels of protein induced parthenogenetic reproduction in some individuals.

The occurrence of significant differences in the amino acid requirement of different larval stages of the silkworm, *Bombyx mori*, has been reported by Stamm and associates (190, 235). The same insect responds favorably to the addition of glycine to mulberry leaves, possibly as a consequence of the large amounts of this amino acid necessary for silk production [Kashiwada (133)]. The ability of other sources of nitrogen to duplicate this effect was not investigated by this worker, but Murthy (194) has observed increased rates of growth and silk production in *B. mori* fed mulberry leaves perfused with ammonium nitrate. Attempts by Stokhovskaya (238) to stimulate the growth of the oak silkworm, *Antheraea pernyi*, by glycine supplementation of oak leaves was unsuccessful. The injection of methionine- S^{35} into this insect is followed by a relatively slow incorporation of isotope into body protein⁵ [Demianovskii *et al.* (51)].

⁵ The authors wish to express their appreciation to Mrs. R. Fraenkel for translations from the Russian.

Vitamins.—The phylogeny of vitamin needs has been discussed by McCay (188). For information on the requirements of stored products insects for known and unknown growth factors see the reviews of Fraenkel (69) and Magis (180, 181). For purposes of this discussion the vitamins referred to as the "major B vitamins" will include thiamine, riboflavin, nicotinic acid, pyridoxine, and pantothenic acid. The tables of Albritton (1) show that these five vitamins are in most cases required by insects which do not harbor symbiotes, and frequently folic acid, biotin, and choline as well. The need for inositol and *p*-aminobenzoic acid has not been demonstrated in insects.

Noland *et al.* (200) have studied the requirements of the German roach reared nonaseptically. The need for the major B vitamins and choline was clearly demonstrated. In the case of *P. americana*, the feeding of antivitamin enabled Siburth & McClaren (229) to show that thiamine, and nicotinic, pantothenic, and folic acids, were required by growing nymphs. It has been found [Pant & Fraenkel (207)] that the intracellular symbiotes of *Stegobium paniceum* and *Lasioderma serricorne* supply these species with riboflavin, niacin, pantothenic acid, choline, biotin, and folic acid. Before sterilization, these insects required only thiamin and pyridoxine in the diet (173, 208). A purified diet has been developed for *Dermestes vulpinus* by Fraenkel (69) on which a retardation of larval growth is manifest following the withdrawal of the major B vitamins, choline, biotin, and folic acid. Stride (239) has reared the dried fruit beetle, *Carpophilus hemipterus*, under aseptic conditions and demonstrated that the larvae require the major B vitamins and choline.

The small eyed flour beetle, *Palorus ratzeburgi*, has been shown to require the major B vitamins and choline for larval growth [Cooper & Fraenkel (42)]. In order for metamorphosis to be completed in a normal manner, carnitine was a necessary supplement. Several laboratories have reported that biotin, folic acid, and the major B vitamins cannot be omitted from the diet of *T. confusum* larvae [Fraenkel (69); Fröbrich & Offhaus (91); Magis (183)]. Choline is in part synthesized by this species [Lemonde & Bernard (174)]. The use of this hardy organism for the qualitative identification of vitamins has been suggested by Fröbrich & Offhaus (91), but the advantages derived from the use of this insect rather than microbiological or chemical methods are not readily apparent. The probability that *Tribolium* requires at least two additional undetermined vitamins, which are present in the water-soluble and insoluble fraction of yeast [Fraenkel (66); French (83); Offhaus (202)] constitutes further reason to view this suggestion with caution [see also the review of Magis (183)]. For the production of normal adults of *T. confusum* and *T. castaneum* carnitine must be added to the vitamins listed above [French & Fraenkel (84); Fröbrich (88); Magis (182)]. The best synthetic diets devised for these species will exhibit an increase in nutritive value following the addition of yeast (183).

Tenebrio molitor cannot dispense with the major B vitamins, biotin, folic acid, and carnitine [Fraenkel *et al.* (76); Leclercq (164)]. The stringent re-

quirement of this tenebrionid for B vitamins indicates that little contribution is made by the intestinal microflora to the nutrition of the insect. The administration of sulfaguanidine and sulfanilamide produces no significant change in the growth rate [Kaudewitz (137)]. That the mealworm required a hitherto unknown growth factor (named B_T) for larval development and the production of normal adults was first shown by Fraenkel and co-workers (76). The isolation and identification of this substance as carnitine (β -OH butyrobetaine) was accomplished by Carter *et al.* (35) and Fraenkel (67). These results have been confirmed by Leclercq (167). In spite of the presence of the methylated quaternary nitrogen in the molecule, carnitine does not donate methyl groups to homocysteine or guanidoacetic acid in birds and rodents [Friedman *et al.* (85); Garkovi (94); Sloan and co-workers (232)]. Carnitine will, however, replace choline in the diet of *Drosophila* [Fraenkel and collaborators (78)] but not in the case of *Palorus ratzeburgi*, *Blatella germanica*, or sterilized *Lasioderma serricorne* (78). The O-acetylation of carnitine by acetyl CoA can be accomplished by an enzyme found in pigeon liver, and the O-acetyl carnitine so formed can acetylate *p*-aminobenzoate or sulfanilamide in the presence of CoA [Friedman *et al.* (86)]. Since Fraenkel & Chang (77) have shown that carnitine-deficient larvae do not mobilize depot fat and also die at the moult, it is possible that deficient larvae are unable to synthesize those components of the new cuticle that require the production and transfer of two-carbon fragments for their formation. In the presence of 0.35 μ g. carnitine most *Tenebrio* larvae survive and pupate, but the resulting adults are malformed and not viable (77) and show exactly the symptoms exhibited by *Palorus* and *Tribolium* grown in absence of carnitine (42, 84, 88). This again shows that cuticle formation and growth is affected. The histopathology of the carnitine deficiency in *Tenebrio* has been described by Chang & Fraenkel (36). The severest symptoms consist of necrosis of the epithelial cells of the mid-intestine. *Tenebrio* larvae have been used to determine the carnitine content of naturally occurring materials by a method developed by Fraenkel (71, 73). This technique has shown that *Dermestes* larvae and *Phormia* maggots synthesize large quantities of the vitamin.

The detailed investigations by Beck (14) into the nutrition of the European corn borer has resulted in the formulation of a diet upon which the larvae may be reared under externally aseptic conditions. In the presence of the major B vitamins, choline, folic acid, biotin, inositol, and *p*-aminobenzoic acid, one or more uncharacterized growth factors present in corn leaf and other plant products were required. Yeast had a better effect on growth than the mixture of the known B vitamins, but growth was still greatly accelerated by the addition of dried corn leaves, grass juice fractions, or wheat germ. The closely related rice stem borer, *Chilo simplex*, will develop on virtually the same basic medium provided the diet contains yeast. Addition of dried rice plant had no further effect in the presence of yeast [Ishii & Urushibara (127)], and diets devoid of yeast but supplemented with dried rice plant failed to support growth of this pyralid [Ishii & Urushibara

(127)]. *Chilo simplex* requires the major B vitamins, folic acid, and probably biotin (127). Vitamin B₁₂ is apparently synthesized by *B. mori* [Sahashi *et al.* (218)].

Viable adults of the pink bollworm, *Pectinophora gossypiella*, may be reared from the egg stage on a semisynthetic regimen containing lecithin, cotton leaf meal, and nucleic acid [Beckman *et al.* (16); Van der Zant & Reiser (257)]. This medium, as well as the crude gel formulated by Coutin (43) for the codling moth, should prove ideal for the further elucidation of the nutrition of lepidopterans.

Significant advances in the study of the food requirements of several dipterans have been reported. Hinton and associates have shown that the wild type of *Drosophila melanogaster*, Oregon-R, develops to maturity on a diet containing the major B vitamins, biotin, choline, and B₁₂ (118). Strains have been developed which require an exogenous source of adenine (117). The effect of purine, pyrimidine, and folic acid antagonists on *Drosophila* has been studied in several laboratories (97, 99, 116, 259). The data show that the antimetabolites exert an inhibitory effect on growth and development that can be reversed by the action of the normal metabolite.

House (123) has shown that *Pseudosarcophaga affinis* requires choline, biotin, and with the rather unusual exception of pyridoxine, the major B vitamins. This ration also contains RNA. An investigation of the vitamin requirements of the onion maggot, *Hylemya antiqua*, has revealed that whereas the male can subsist on the major B vitamins, choline, biotin, folic acid, and RNA, the female requires the additional feeding of B₁₂, CoA, and lipoic acid [Friend & Patton (87)].

A study of the nutrition of the adult female blowfly by Rasso & Fraenkel (214) demonstrated that potassium, phosphorous, choline, and nicotinic acid stimulated ovarian development, providing that sugar and a water soluble protein were available to the organism [Also see Allegret (2); Legay (170)]. The best synthetic diet, however, still proved greatly inferior to fresh liver for *Phormia regina* (214). The vitamin needs of the larvae of the same species of blowfly, *P. regina*, include the major B vitamins, choline, and folic acid when the animals are reared aseptically [Brust & Fraenkel (31)]. It was further shown by these workers that growth of this species, and *Lucilia* (*Phoenicia*) *sericata* as well, was stimulated by the presence of yeast in the diet, but adenine, uracil, guanine, DNA, and RNA could not replace the yeast. An earlier report on *Lucilia* larvae by Kadner & LaFleur (131) had shown an exogenous source of the major B vitamins to be necessary for the maggots. Another blowfly *Calliphora erythrocephala*, has been reared in axenic culture, under which conditions the major B vitamins, choline, inositol, biotin, and folic acid support maximum growth [Sedee (226)].

By feeding the folic acid antagonist, aminopterin, Mitlin and co-workers (191) demonstrated a requirement for the vitamin in the house fly, *Musca domestica*, and recorded that this phenomenon was reversible by folic acid. The same investigators have tested the action of other antimetabolites on

the house fly with some success as far as growth retardations are concerned, but reversal attempts are not reported (151). Bhattacharyya and co-workers (22) have demonstrated a reversible competition between carnitine and butyrobetaine in *Tenebrio*. The administration of the ascorbic analogue, D-glucosascorbic acid, was without deleterious effect on the metabolism of *B. germanica** [Day (47)].

The feeding of heavy doses of antibiotics resulted in high mortality rates in the German roach [Liles & Fisk (176)] and various pests of stored grains [Steinhaus & Bell (236)]. The authors suggest that certain enzymes present in the insects are inhibited in a manner similar to the action of these substances on bacterial systems. On the other hand, low doses of antibiotics are known to stimulate the growth of higher animals [see Braude *et al.* (28)], and this effect has been extended to the silkworm by Murthy and associates (195). When chloramphenicol (chloromycetin) was added to diet of mulberry leaves sprayed with casein hydrolysate, a significant increase in body weight, fertility, silk production, and disease resistance was manifest.

A vigorous branch of the study of insect nutrition is the school concerned with the metabolism of tryptophan and niacin in insects. The inability of tryptophan to replace niacin in the diet of *Drosophila* was first reported by Schultz & Rudkin (225). This phenomenon now includes *T. molitor* and *T. confusum* [Fraenkel & Stern (80)] and *P. regina* (31). This finding was to some degree unexpected, since several of the intermediates, e.g., 3-OH-kynurenine (3-OHK) and 3-OH-anthranilic acid (3-OHAA), which are formed in the conversion of tryptophan to niacin in vertebrates and microorganisms are known to occur in insects [see Bourne & Kidder (27)]. In the case of hexapods, these intermediates are required for the synthesis of eye pigments [see Butenandt *et al.* (33); Kikkawa (143)] or cuticular tanning reagents [Pryor (212)]. Only in the case of the rice moth larvae, *Corcyra cephalonica*, has it been clearly established that niacin is synthesized via 3-OHK, which in turn is derived from dietary tryptophane [Sundaram & Sarma (241, 242)]. In the case of the silkworm, Kato (134) has reported that the enzymes necessary for this conversion are present in the caterpillars, but Kikkawa has questioned the reliability of the analytical methods used (143). Kato & Hamamura (136) have obtained evidence of niacin synthesis from 3-OHK in the pupal stage, but their results are in turn contested by Ito (128). The latter observer concluded that niacin synthesis in the pupa proceeds by a different route than occurs in mammals and *Neurospora*, and the decrease in tryptophan observed in the pupal stage only marks the synthesis of indole pigments. This evidence is based on the observation of active niacin synthesis in mutants blocked at the kynurenine \rightarrow 3-OHK stage.

The studies of the Indian school on the role of pyridoxine in the tryptophan-niacin relationship represent insect nutrition in its most fascinating

* This has also been confirmed for *Tribolium confusum* [Fraenkel (Unpublished data)].

aspect. Recognizing the role of pyridoxal phosphate in the enzymatic conversion of 3-OHK to 3-OHAA [Dagleish (45)], it was observed by Sundaran & Sarma (240) that pyridoxine-deficient *Corcyra* larvae, reared in the presence of tryptophan, excrete fecal pellets tinted yellow with 3-OHK. Furthermore, the deficient animals were unable to synthesize nicotinic acid unless pyridoxine was added to the diet. Further studies on niacin metabolism, contrary to earlier findings in *T. molitor* by Ellinger *et al.* (62), have shown that this vitamin is not excreted in the methylated form in *Corcyra* (243) or in *Bombyx* and *Lucilia* (135). Another series of nutritional contributions by Sankar & Sarma (220) have implicated biotin in the role of a cofactor for the oxidative deamination of amino acids in *Corcyra*.

A nutritional requirement for vitamins other than those of the B-group have never been clearly substantiated. Goodwin (100) in an exhaustive review of the occurrence of carotenes in insects and other animals states that carotenoid pigments are always derived from vegetable food and that synthesis of vitamin A from carotenes never occurs in insects. Murthy, however, working with the silk worm *Bombyx mori* claims that the addition of carotene and ascorbic acid is of benefit on a basic diet consisting of mulberry leaves extracted with alcohol and benzene (194). Yoshida (267) has studied the utilization and absorption of chloplast pigments in the silkworm and has shown that some conversion of carotene or other provitamin A into vitamin A occurs. He describes synthesis of proline from the ingested chlorophyll and proposes some physiological function of carotenes and chlorophyll in the metabolism of the insects.

Sterols.—No exception has been found to the rule that insects require a dietary source of sterol. The carion eating larvae of *P. regina* can utilize phytosterol and other sterols of animal origin [Brust & Fraenkel (31)], thereby differing from other insects which subsist naturally on foods of animal origin [see Trager (253)]. An extremely thorough series of investigations by Noland (196, 197) concerning the sterol requirements of *B. germanica* have revealed that sterol derivatives whose structural topography is such as to inhibit cholesterol esterase, also blocks cholesterol utilization. Thus the data indicate that sterols are esterified in the course of absorption. Both the cowpea weevil, *Callosobruchus chinensis* [Ishii (125)], and the German roach can dispense with the double bond at position 5 of cholesterol but in keeping with the hypothesis proposed by Noland require the hydroxyl at carbon 3. These papers as well as those by McKennis (189) on *Attagenus piceus* and by Silverman & Levinson (231) on *Musca vicina* contain extensive lists of the nutritive value of sterol derivatives for the insects in question that are not readily integrated into a unified theory. Chauvin (38) has shown that the sterol requirements of *Gryllus domesticus* and the German roach are similar. Finally, Sarma (221) has reported a sharp increase in the titer of digitonin-precipitable substances following the feeding of benzene hexachloride to *Corcyra*. The possible relationship between inositol and this insecticide are discussed by Kearns elsewhere in this volume.

Carbohydrates.—Albritton's tables present data relating to the utilization of 40 different carbohydrates by insects (1). The older literature pertaining to sugar requirements of *T. confusum*, *C. erythrocephala*, *P. regina*, and *D. melanogaster* and other species is discussed by Trager (253) and Dethier (53). The Mexican fruit fly, *Anastrepha ludens*, has also been studied recently by Baker *et al.* (8) as well as *M. domestica* and *Aedes aegypti* (92). Much of the older literature dealing with the effect of carbohydrates on growth and survival is not readily interpretable, since it has only recently been recognized that sugars may prove to be inadequate in a dietary sense for at least three reasons. A given carbohydrate may be (a) inert nutritionally, (b) satisfactory as a carbon source but unacceptable in a gustatory sense, or (c) toxic, per se (55, 74 92).

An ingenious method for the exact determination of the quantity of soluble material imbibed by insects has been developed by Dethier & Rhoades (55). The survival value of various sugars for adults of the parasitic hymenopteran, *Macrocentrus ancyliivorous*, shows glucose, maltose, sucrose, and fructose to be well utilized, lactose was unsatisfactory, and galactose was intermediate between the two groups [Pielou & Glaser (211)]. Ribose, galactose, mannose, cellobiose, and α -methyl glucoside failed to support the growth of *Stegobium paniceum*, and the pentoses xylose and arabinose were also unsatisfactory [Lemonde & Bernard (173)]. These workers also studied sugar utilization in *Oryzaephilus surinamensis* and observed no larval growth in the presence of the above three pentoses, α -methyl glucoside, dulcitol, inulin, or sorbose. An extensive list of carbohydrates capable of supporting good rates of growth in these insects is also presented. Tsutsi & Saito (254) report that *Chilo simplex* cannot utilize starch during the first instar, but later stages may do so.

Fraenkel (74) has compared the food value of various sugars with the relative presence of absence of the appropriate carbohydrate-hydrolyzing enzyme in the digestive tract of *Tenebrio*. In general the ability to split a given oligosaccharide was a strong indication that the carbohydrate was suitable for growth. Similarly, a good correlation was established between utilization of an oligosaccharide, polysaccharide, or glucoside and the presence of the appropriate hydrolyzing enzyme in *M. domestica*, *Sarcophaga bullata*, and *Aedes aegypti* adults [Galun (92)]. Although numerous reports can be found concerning the nonutilization of various sugars by insects [see Dethier (53); Trager (253)], some sugars and sugar alcohols exert a strikingly toxic rather than a purely inert effect on the mealworm (74). One part of α -methyl glucoside, for instance, blocked the utilization of approximately 80 parts of glucose, xylose, ribose, or dulcitol, and α -methyl and mannoside had a similar, if slightly lesser effect. The suggestion is made that the toxic carbohydrates interfere in some manner with the absorption or oxidation of those sugars which normally are well utilized. Galun (92) has shown that only by accurately measuring food intake is it possible to distinguish between inert, repellent, and toxic carbohydrates. It was shown in

tests with *Musca domestica* and *Sarcophaga bullata*, using the method developed by Dethier & Rhoades (55), that in general, the simple sugars which failed to support life were repellent, and to a lesser or greater extent toxic. This was especially so in the case of the pentoses xylose, arabinose, ribose, and rhamnose, and also with inositol and α -methyl mannoside. Pentoses or pentosans may inhibit growth in *C. simplex* (126).

Miscellaneous papers.—Gerpe & Guelbenzu (96) have used spectroscopic methods to compare the mineral content of mulberry leaves and mulberry-fed silkworms. A direct correlation is claimed between the presence of silver in the leaf and the ability to support maximum growth in *B. mori*. Analysis of many more samples of diverse origin, as well as studies with plants grown on a medium whose mineral content is accurately known, are required to substantiate this finding. The hormone like substance present in royal jelly which is required for the production of honey bee queens has been shown to deteriorate rapidly at 1°C. in an atmosphere of CO₂ [Weaver (263)]. A marked toxicity has been claimed for saturated free fatty acids of chain length C₆ to C₁₂ for maggots of *Musca vicina* [Levinson & Ascher (175)]. Feeding trials with esterified fatty acids are needed to determine whether this effect is gustatory or toxic per se.

The quantitative determination of food intake, digestibility, assimilation coefficients, and other indices of food utilization continue to be a much neglected phase of insect nutrition, especially in the rather obvious case of purified diets where high orders of digestibility are assumed. The use of species whose fecal pellets are well-formed and the availability of markers and sensitive new methods of analysis should yield much valuable information of this nature. Lafon has prepared an excellent review of the limited information now available (157, 158).

By repeatedly weighing food, feces, and silkworm, Legay (170, 171) showed that in each instar food consumption rises to a maximum and then falls rapidly to a more or less steady state [also see Malucelli (186)]. Since, in these studies, the introduction of fresh leaves stimulated food consumption, similar experiments are in order where the shape of the author's "satiation curve" is determined when only fresh leaves are fed. Such a procedure would compensate for any changes in leaf palatability in the course of an experiment. A study of the quantity of food assimilated by first and fifth instar *Schistocerca gregaria* showed 78 per cent assimilation by the young nymphs and but 38 per cent for the older individuals [Davey (46)]. The provision of an index of the relative digestibility of the diets fed the two groups of locusts would add greatly to the value of the data.

Symbiosis.—The experimental and morphological aspects of symbiosis are presented in an excellent book by Buchner (32). Reviews and discussions on many aspects of symbiosis are contained in the report of a U.N.E.S.C.O. symposium on "Symbiosis of Insects with Microorganisms" held in Amsterdam (162). Some of the genetical and evolutionary aspects have been discussed by Lederberg (169).

The work of Pant & Fraenkel (207) with *Stegobium* and *Lasioderma* has already been discussed under vitamin requirements. Larvae reared aseptically were shown to require seven B vitamins for growth, whereas prior to sterilization, only two such growth factors were necessary. The symbiotes were isolated, subcultured, and identified as yeasts. The yeasts of the two species of insects were morphologically characteristic and easily distinguishable. Sterilized insects have been successfully reinfested with their own yeast and also with those yeasts isolated from the other beetle species. The yeasts retain their morphological and physiological characteristic in the foreign host. The yeasts also provide the insects with a source of sterols. Gräbner (101) reported the isolation and culture of the symbiotic yeasts of the same species and several other representatives of the Anobiidae and Cerambycidae, and recorded interesting differences in the metabolism of yeasts isolated from different insect species. In pure culture the yeasts of *Stegobium* release thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, folic acid, and biotin into the medium. These are precisely the vitamins with which the insects with symbiotes can dispense (207). This suggests that in the normal course of events vitamins from the symbiotes reach the host by diffusion from the mycetome. The dietary effect of symbiotic yeasts, when added as a dry powder to the diet of sterilized *Stegobium* and *Lasioderma* is similar to that of brewers yeast (207). Hennies (108) has discussed the relation of various yeast genera to insects.

Wigglesworth has discussed the occurrence of symbiotes in blood sucking insects (266). Symbiotes are found in those forms whose diet is restricted to blood, a food stuff normally sterile as well as deficient in vitamins and carbohydrates. On the other hand, hematophagous insects whose diet contains other materials which may provide growth factors and a source of energy are devoid of symbiotes. Additional evidence for these generalizations has been supplied by Puchta (213) who sterilized the eggs of the clothes louse, *Pediculus vestimentis*, and reared the nymphs on a ration composed of whole blood plus the major B vitamins and folic acid. Withdrawal of each of the micronutrients, with the exception of thiamine, resulted in a cessation of growth. The symbiotes also were shown to provide the parasite with a factor for the production of fertile females. Geigy and co-workers (95) have cultured the symbiotes of *Triatoma infestans* and report the elaboration of thiamine, folic acid, choline, inositol, *p*-aminobenzoic acid, and B₁₂ into the medium. Some, but not all of the symbiotes may be transferred from *Triatoma* to sterile individuals of *Rhodnius prolixus* in order to provide the latter species with the necessary micronutrients for development [Bewig & Schwartz (21)].

Two strains of the granary weevil, *Sitophilus granarius* exist in the Old World (32). The Egyptian strain apparently contains no microflora of importance nutritionally, but the European strain is known to harbor symbiotes. Schneider (224) has shown that if the European strain is reared at high temperatures, a form is produced with degenerated mycetomes such as occur in

the Egyptian variety. The tropical grain pest, *Rhizopertha dominica* also contains symbiotes which can be eliminated at a culture temperature of 38°C. [Huger (124)].

Fink (65) found that under the combined effect of starvation and high temperature the symbiotes of the mealybug, *Pseudococcus citri*, degenerated. The consequences were similar to those reported for lice, a degeneration of the ovaries and premature death of the bugs. The dry substance from a pure culture of the symbiotes contained the vitamins which occur in aqueous yeast extracts. This, and other published and unpublished work of the Munich school has been reviewed by Koch (146 to 149).

The rapid development of many insects on foods of apparently low nitrogen content has resulted in a widespread interest in the possible role of symbiotic microflora in the fixation of atmospheric nitrogen. New experimental evidence in favor of this hypothesis is restricted to the studies of Tòth (250) who observed that breis of termites and various homopterans were capable of the reduction of atmospheric nitrogen in the presence of certain dicarboxylic acids as a carbon source. The fixation would not occur unless the nitrogen content of the medium was kept at very low levels. It was also claimed that these insects conserve organic nitrogen by the re-use of nitrogenous excretion products on the part of the symbiotes (251). Wigglesworth [see under Tòth (250)] has stressed the absence of direct evidence for these processes in the form of balance studies and experiments with isotopic nitrogen, while Fraenkel (70, 250) questions the ability of the microflora to fix atmospheric nitrogen in the nitrogen-rich milieu of the insect cell. In spite of these criticisms, the attractiveness of the Tòth hypothesis continues to bring forth reports of nitrogen fixation in insects and related forms [Balogh & Gere (9); Bewig & Schwartz (21); Beerstecher *et al.* (18)].

The subtle interrelationships of the symbiotic condition have provoked still another controversy. The body cavity of aphids contains cells which at certain times become filled with dark globules resembling microorganisms or large microchondria. Carrère has reported the successful cultivation of these structures from *Siphonophora rosae* and states that they are definitely symbiotic microorganisms (34). The majority of attempts to culture these bodies, however, have proven unsuccessful. A histochemical investigation by Lanham (161) showed that these bodies do not stain with acid Giemsa, a reagent which would normally color bacterial DNA, and it is suggested that the objects are mitochondria. Trager (252), however, questions the reliability of the stain, since other microorganisms known to contain DNA do not respond to the test, and also emphasizes the need for nutritional investigations into this problem. Peklo (208a) is similarly critical of Lanham's conclusions. General papers of interest include a contribution by Perez (209) on the culture of symbiotes of insect origin, and another by Vago (255) on techniques available for the sterilization and inoculation of insect eggs. A report by Clayton (41) calls attention to the differences which may occur in the protozoan populations of termites following artificial as contrasted to natural

(i.e., postecdysial) refaunation of sterilized colonies.

Fraenkel (70), Buchner (32) and Koch (148) have reviewed existing evidence to the effect that symbiotes serve as sources of vitamins and other growth factors for their insect hosts. This hypothesis does not pretend to explain all cases of symbiosis but seems to account for the general occurrence of symbiosis in three important groups of insects, those feeding on blood, on wood, and on plant sap. Blood is known to contain thiamine, riboflavin, pantothenic acid, and pyridoxine in quantities far below the levels required by insects investigated in this respect, but blood is adequate in nicotinic acid (70). Concerning the two other foods we are on less secure ground. The presence of vitamins in wood has never been reported, but their occurrence in nutritionally significant amounts seems highly improbable. The paucity of vitamins in the phloem sap of oak has recently been reported (148).

THE NUTRITION OF INSECTS FED NATURAL DIETS

Insects which feed on blood.—Downe & West (57) have discussed the use of the precipitin test for host determination in hematophagous and predacious insects. A comprehensive volume on the bionomics of mosquitoes lists references to many nutritional techniques and investigations [Horsfall (121)].

Considerable study has been devoted to the nutrition of autogenous races of mosquitoes. These strains are capable of the production of viable eggs without the benefit of a blood meal, thus posing the question of the origin of the nitrogenous components of the embryonic tissue. Hocking (120) states that histological examination of gravid females reveals that autolysis of the flight muscles occurs in the autogenous species, *Aedes communis*, and the resulting degradation products are incorporated into the egg. On the other hand, Beckel (15) denies that such a process is operative in this species. Histochemical evidence has also been offered to the effect that the autogenous strain of *Culex pipiens* accumulates protein in the fat body which is mobilized during ovarian development [Bettini (20)]. This process was not observed to occur in anautogenous species. The production of eggs by anautogenous species on crude diets devoid of blood is reported by Lea *et al.* (163).

In *Culex pipiens* digestion of erythrocytes may be accomplished by symbiotes [Arnal (5)]. The digestive process in the louse *Pediculus humanus* has been studied by Krynski and associates (154) in an effort to elucidate the toxicity of guinea pig blood to this parasite. It was observed that the intake of a blood meal was followed rapidly by the formation of crystals, apparently hemoglobin, in the gut. These crystals accumulated until the intestinal epithelium was ruptured and death of the louse occurred. No explanation is offered for the absence of this phenomenon following feeding on hosts other than the guinea pig.

A blood meal is required by males of *Rhodnius prolixus* for the production of viable sperm, but not by males of the bed bug *Cimex lectularius* [Khalifa (141)]. The fertility of *Cimex* females is directly proportional to the

number of blood meals partaken. According to Sharif (228) various flea larvae of the genus *Xenopsylla* which live as scavengers until maturity, require an added source of vitamins when fed blood alone (see under symbiosis). Iron was shown to be required for proper pigmentation of the larval cuticle. Rats depleted of choline, folic acid, or pyridoxine have been reported to lose their parasitic anoplurans [Kartman (132)]. Data pertaining to the changes in both the vitamin content of the blood levels of the host and also the tissue of the parasite have been noticeably lacking in studies of this nature.

Honeydew secretion and nutrition of Homoptera.—The high order of fecundity in homopterans, especially aphids, with its pronounced demand for the elaboration of embryonic nitrogen has stimulated inquiries into the adequacy of plant sap as a source of protein for sucking insects. The alleged deficiency of plant sap in nitrogen is generally considered as the cause of the insects ingesting vast amounts of sap and excreting the surplus sugars as honeydew.

The role of symbiotes in these phytophagous forms has been discussed in previous sections. Although the phloem sap has generally been presumed to contain only small quantities of nitrogen, Wigglesworth & Fraenkel (250) in rebuttal to a paper by Tóth (250) have gathered evidence which shows that the phloem sap may contain more than enough nitrogen for the needs of a parthenogenic insect. The development of ingenious methods for the collection of plant fluids should yield more data concerning phloem sap analysis [Kennedy & Mittler (140); Ledbetter & Flemion (168)]. The volume of food imbibed by homopterans may be quantitated by radiometric techniques [Day & Irzykiewicz (48); Day & McKinnon (49); Watson & Nison (262)] and a new method based on measurements of virus transmission⁷.

Lindemann (177) in a comparison of the nitrogen content of honeydew produced by the aphid, *Cryptomyzus ribis*, and the phloem sap of its host, *Ribes rubrum*, found the sap always to contain higher quantities of nitrogen. According to Mittler (192), the honeydew of the willow aphid contains less total nitrogen, but the identical amino acids as the phloem sap from which it was derived, further indicating that nitrogen fixation did not occur. No quantitative difference was observed between the amino nitrogen of sap and honeydew after acid hydrolysis; according to Mittler (192) sap protein was not utilized by the aphid. In the case of the pineapple mealybug, *Pseudococcus brevipes*, the honeydew did contain amino acids not present in juice pressed from the leaves, but the author believes that the newly-formed amino acids may be the result of the activity of symbionts not necessarily concerned with nitrogen fixation, or possibly waste products of excretion [Gray (103)]. The number of amino acids in the honeydew of *P. brevipes* increased from four after one day of feeding to 13 after 10 days, but this observation may reflect the extent of tissue histolysis accompanying moulting of the insects. It was also observed that the honeydew varied in composition according to

⁷ Personal communication from Dr. R. H. Bradley, Division of Entomology, Fredericton, New Brunswick, Canada.

the part of the plant serving as food. Johnson (130) has observed a loss in the ability to fly by females of *Aphis fabae* during the production of living young, which on microscopic examination appeared to be attributable to autolysis of the flight muscles.

Two independent studies on the carbohydrate components of honeydew have corroborated one another in a manner gratifying to both groups of investigators. Duspiva (58) compared the sugars in the sprouts and stems of the apple tree with the honeydew excreted by *Aphis pomi* and the woolly apple aphid, *Eriosoma lanigerum*. The two materials were virtually identical with respect to their carbohydrate content, except for the presence of α -maltosyl fructofuranose which occurred in the honeydew only. While feeding on potato the citrus mealy bug, *Pseudococcus citri*, was observed by Gray & Fraenkel (102) to excrete what was probably the same trisaccharide. They also observed the identity of the sugars occurring in the host plant and the honeydew. Both groups of investigators attribute the appearance of the trisaccharide to transglycosidation occurring in the insect gut, although the possibility exists that this exchange may actually be mediated by intestinal or microbial enzymes acting after excretion of the honeydew. The formation of new oligosaccharides from nectar sugars has been shown to occur by the same exchange mechanism in honey [Taufel & Müller (244); White & Maher (264)]. The demonstration that transglycosidation occurs in the gut of insects would furnish some evidence for the contention that the sugars of the cell sap are actually digested and metabolized rather than merely flushed through the digestive system in the course of the insect's quest for nitrogen.

Host preference in phytophagous species.—The current revival of interest in this field, although gratifying, falls far short of the emphasis that the importance of this subject warrants. Constituting as it does the very heart of agricultural entomology, the study of host preference has received relatively little attention from the very institutions that possess the physical and financial means to pursue this problem to a satisfactory conclusion (215).

Philosophical aspects of host selection are treated by Dethier in this volume and elsewhere (52, 54). Further insight may be gained by several reviews of varying length [Thorsteinson (247); Heron (110); Painter (205, 206); Rubtsov (216)]. Methodological aspects are discussed by Thorsteinson (249) and should be greatly aided by a new series devoted to the chemical analysis of plants (204). Several of the more important papers to be discussed constitute part of a symposium devoted to host specificity (52, 68, 138, 206).

One main branch of insect control in agriculture deals with the production of resistant plants. The fragmentary state of our knowledge of the role of nutritional factors in resistance can be seen from the important monograph by Painter (205) which out of 520 pages devotes only one page to this topic. That physical factors of plant structure such as the presence of spines or a thickened leaf cuticle may be involved in plant resistance to insects has long been recognized (19, 142, 145, 205, 230, 258). Although not ac-

accompanied by physical measurements of shear strength, the data of Sasamoto (222) indicate that high levels of silicon in the soil and consequently in the rice plant increase resistance to the rice stem borer by hardening the tissues.

The most fundamental aspect of host selection in leaf eating species inquires whether the selection is governed (a) by the nutritional superiority of the plant or region of the plant serving as food for the insect, or (b) by the presence or absence of attractants and repellents in plants of more or less uniform food value to which the parasitic species has become adapted. The latter view implies that the token stimuli, i.e., the substances which are responsible for the acceptance or rejection of the host, have no nutritional value for the insect in the sense that they are metabolized and incorporated into the host tissue or biochemically involved in tissue synthesis. They may be viewed as trigger compounds which induce the uptake of true nutrients. In fact, Thorsteinson (248) has shown that these token stimuli exert their maximum sensory action on a ration of maximum nutritive value.

To deal first with proposition (a) in his studies of host plant selection by *Aphis fabae*, Kennedy (138, 139) has observed two factors to govern the elicitation of a feeding response. The first consists of the insect's ability to discriminate between different species of plants, whereas the second is expressed as the ability of the aphid to choose a leaf whose maturity has reached the stage most acceptable and most nutritious to the parasite. The first factor, governing botanical choice, probably is controlled by the distribution of token stimuli. The second, and most controversial factor, infers the presence of a mechanism capable of differentiating between leaves of different ages to the extent that the most nutritious is chosen as food. In this latter case the token stimuli are presumed to be either identical with the nutrients or very closely associated with them. While undoubtedly elegant in its conception, this dual discrimination theory is unaccompanied by data relating the quantities of food partaken by aphids feeding on leaves of various stages of maturity, and leaf analyses are restricted to a brief report from this laboratory that the free amino acid content of the phloem sap is highest during the stages most acceptable to aphids [Mittler (192)]. It was also observed that galled or virus infected leaves are preferred by the aphids, and it is argued that these invasions accelerate the conversion of the leaf to the stage most acceptable to *A. fabae* and other species [Arenz(4); Kennedy (139)]. An equally tenable hypothesis would assume the token stimuli to be metabolic products of the leaf during periods characterized by rapid cell proliferation or autolysis or both, thus the chemicals eliciting the feeding response would be inseparably linked to the stage most nutritious to the aphid. In the case of the latter proposal it would be the token stimuli which would govern the insect's choice, the rise in nutritive value would be coincidental.

Fennah (64) has stressed the correlation between the feeding activity of homopterans and the metabolic activity of the plant. This investigator also

believes that the rise and fall of soluble nutrients govern acceptability, but it is again possible that this fluctuation in plant sap constitution is but a reflection of deep-seated chemical changes at the cellular level of plant tissues. It follows that such cytological transformations could in turn govern the release or withdrawal of compounds which induce feeding. Painter (206) and Lambers (159) re-emphasize the simultaneous development of the major phytophagous families of insects with the evolution of more chemically complex plants, and Hering (109) believes that geographical redistribution of a plant species eventually alters the chemical constitution of the leaf to the extent that it is rendered unacceptable to former parasites.

The existing data pertaining to the food value of green leaves have been reviewed by Fraenkel (68), who concludes that sufficient levels of all the major nutrients are present in most, if not all leaves to satisfy phytophagous insects. Thus the statement that insects consume those plants that best satisfy their nutritional demands is not supported by chemical analysis. The possibility also exists that the diet of some leaf eating insects is more diversified than previously suspected. The widespread application of insecticides to the soil has revealed that many species previously supposed to exist only on the aerial regions of the plant do in fact journey frequently to the ground where a host of new dietary materials is available. It can also be argued that insufficient analytical data exist to support the contention that seasonal changes effect nutritive value to the extent that host selection can be explained on this basis (68). As an alternative hypothesis, Dethier (54) and Fraenkel (72) offer rejuvenated versions of the theories of Stahl, Lagerheim, and Verschaeffelt [see Fraenkel (68, 72)], namely, that many constituents of leaves whose function is unknown serve as repellents to insects in general, and probably as attractants to those few insect species that feed on each plant species. This proposal, a restatement of proposition (b) (see p. 32), would account for the irregular distribution of the curious glycosides, tannins, saponins, alkaloids, and essential oils whose occurrence is unexplained by plant physiologists. It is contended that early in their evolution plants developed the means by which they became unpalatable to the rising multitude of insects. The unpalatability was in turn accomplished by the production of the vast array of chemical compounds which now characterize specific taxonomic groups of plants. In fact, the appearance of flowering plants in the early Cretaceous coincides with the development of the various morphological and physiological adaptations in insects which characterize the interdependence of insects and the flowering plants (72, 159, 206). Fraenkel (72) and Dethier (54) hold that host preference begins when a given insect species, by genetic selection, overcomes the repellent effect of such a material, thereby gaining a new source of food and resulting ideally in a situation where further genetic selection produces strains of the original species who require the former repellent (now the attractant) to induce feeding.

The role of secondary plant substances is strikingly illustrated by the

recent studies of Thorsteinson (248) on the direct correlation between the presence of the mustard oil glycosides and the feeding response of the cabbage pest, *Plutella maculipennis*. Artificial diets, as well as normally unacceptable hosts could be made to elicit a feeding response by the addition of the glycosides, sinigrin and sinalbin which are normal constituents of the Cruciferae. Other pests of the crucifers have been shown to follow essentially the same pattern of host selection as *Plutella* [Johansson (129); Markkula (187)]. In the case of bark beetles, Ohnesorge (203) has shown that although various sugars differ markedly in their ability to elicit a sustained rate of feeding, it is the extractable token stimuli, not the carbohydrates per se that inaugurate the feeding process. The Colorado potato beetle, *Leptinotarsa decemlineata*, is believed by Hesse & Meier (111) to be attracted to various Solanaceae by naturally occurring aldehydes, but Langenbuch (160) was unable to substantiate these findings, and Chauvin (39) has fractionated the leaves of this family to produce an attractant not related structurally to the compounds isolated by the German workers [also see Kuhn & Low (156)]. Chauvin has also prepared a review of the chemistry of attractants (37). An extremely profitable approach to the study of host preference is suggested in passing by Fennah (64), who is studying this phenomenon by means of scion-stock grafts of accepted and rejected varieties of plants.

Evidence contrary to proposition (b), namely that token stimuli are not necessarily involved in host selection, consists of the following: In those varieties of legumes acceptable to the cowpea weevil, *Callosobruchus chinensis*, such as the adzuki bean, *Phaseolus angularis*, choice is determined by the smoothness and curvature of the bean. Unacceptable varieties, however are rendered so by the presence of repellent compounds [Ishii (126)]. In these studies repellency was investigated by offering the beetles artificial beans made of flour from different varieties or mixtures thereof, and also by the addition of fractions isolated from unsuitable varieties of beans. The contention by the author that pentosans in *P. vulgaris* may be repellent or toxic does not seem to be borne out by the facts, since the pentosan contents of acceptable and nonacceptable varieties do not differ markedly, and furthermore the hydrolysis of pentosans in the intestine has not been demonstrated.

The banana weevil, although attracted to the host by olfactory stimuli, requires no taste factors to maintain its initial rate of feeding [Cuille (44)]. Several investigations concerning the importance of leaf maturity have shown that immature leaves are better able to support the growth of insects than mature ones [Emery (63); Fraisse (81, 82); Grison (104); Kozhanchiko (152)], but these studies fail to present evidence that the older leaves do not accumulate toxic or repellent compounds. Maltais has analyzed a limited number of varieties of peas and reported higher rates of water-soluble nitrogen to occur in aphid-susceptible varieties (185). Most challenging of all is the report by Garber (93) that the use of whole homogenates of plants for tissue analysis may give an erroneous picture of nutrients available to parasites subsisting in localized areas of the host. These results were ob-

tained by the ingenious use of amino acid mutants of bacterial pathogens.

The immunity, as well as the proclivity of plants to insect attack may also be put on a chemical basis. Lipke and co-workers (178) have shown that the soybean, a plant whose freedom from insect damage in the United States is generally acknowledged, contains substances which are toxic to insects in low dosages. It was shown that the beans contain a heat labile substance, probably a protein, that inhibited proteolysis in *T. confusum* in addition to exerting a toxic effect by other, unknown routes. The toxin was distinct from those implicated in the growth inhibiting effect of unheated soy on vertebrates. Evidence of the same nature was also offered for the purpose of explaining the inability of *Tenebrio* to mature on corn rations [Lipke & Fraenkel (179)]. In the latter report, a toxic protein was shown to occur in the germ region of the maize kernel which was also different from those substances responsible for the deleterious effect of corn in the diet of vertebrates.

The host preference of *L. decemlineata* to many species of Solanaceae provides a striking illustration of the role of token stimuli in the resistance of plants to predation. The ability of this insect to develop on the common potato, *Solanum tuberosum*, is a matter of grim public record, but *S. demissum*, *S. chacoense* (a wild potato), and many other members of the family are avoided or fail to support growth of the beetle [see Langenbuch (160); Schaper (223)]. Substances present in *S. demissum*, possibly the glycoside demissin, inhibits ovarian development in the females [Boczkowska (25); Schaper (223)]. A similar phenomenon may explain the resistance of certain varieties of pear to *Anthonomus piri* [Kuenen & Leins (155)]. Among the Solanaceae again, the genus *Nicotiana* is highly resistant to the potato beetle with the exception of those species containing unusually low amounts of the nicotine alkaloids (Koenig & Koelle (150)). Removal of the site of alkaloid synthesis in the Solanaceae, namely the subterranean regions of the plant, renders previously rejected varieties of potato acceptable to *Myzus persicae* [Arenz (4)].

The unsuitable nature of *S. chacoense* to the potato beetle appears to be a result of the presence of repellent or toxic compounds, rather than the absence of an attractant. Sap from the common edible potato did not stimulate feeding on *S. chacoense*, but leaf extracts from the latter plant caused *S. tuberosum* to be rejected by the insects [Langenbuch (160)].

This report is at variance with a previous paper by Hesse & Meier (111) which states that *S. demissum* became acceptable to the potato beetle after infiltration of the leaves with press juice from *S. tuberosum*. The dissenting results of Langenbuch are corroborated by Schaper (223) who also observed the production of infertile females on hybrids of the common and the wild potato. The degeneration of the ovaries of *L. decemlineata* is frequently attributed to the presence of a toxin (25, 223), but since no data are presented with respect to food consumption it is possible that starvation is the causative agent of the ovarian atrophy. Chin (40) believes that both factors

may operate in the Solanaceae, starvation occurring on most resistant species and intoxication on petunia and belladonna. This contribution offers evidence to the effect that the acceptance of the plant depends on the presence of suitable olfactory stimuli which elicit a biting reaction on the part of the insect. This biting reaction occurs on many solanaceous plants even though the host is soon rejected as a food or even proves to be toxic following continued ingestion. A higher degree of sensitivity to the substances causing resistance was observed in first instar larvae than in later instars by this investigator (40). Before concluding this section of the potato beetle and its host, it should be mentioned that the mass of conflicting data available indicates the need for an integrated research program, including a thorough verification of existing reports.

The above mentioned variation in discriminatory ability during larval life has been observed to include adulthood in the case of *Bruchus maculatus* subsisting on legumes [Zaazou (268); El Sawaf (60)]. It is also reported by El Sawaf (61), that ovipositing females of this weevil show increased fecundity when ovipositing on previously infested hosts [also see Barnhart & Chadwick (13)], but Zaazou believes that undamaged peas are superior oviposition sites (269).

The concept that larval feeding habits strongly influence oviposition choice is discussed by Dethier (54) in his consideration of the Hopkins Host Selection Principle. Although subject to great variation among species, individual races of a given species may conform quite strictly to this rule [Azab (7); Bremer (29)]. The adaptability of insects to strange hosts has received considerable attention from Russian workers interested in the experimental demonstration of the inheritance of acquired characters [Samokhvalova (219); Kozanchiko (152, 153); Tazima (246)]. The oak silkworm, *Antherea pernyi*, is reported to prefer the strange host birch, to the natural host oak, following the hybridization of oak-reared individuals with those birch-fed insects that survived feeding on the strange plant [Averkief (6); Vanderlass (256)]. A similar transformation is claimed for parthenogenic insects by Smirnov & Chuvakhina (233), who produced races of *Neomyzus circumflexus* favoring the foreign host radish, over vetch. The scale, *Eulecanium corni*, when removed from mountain ash, easily adapts to ivy, but when the reverse is attempted with supposedly the same species of scale, no multiplication is manifest [Rubtsov (217)]. This author states that the "adaptability" of scale species to strange hosts may differ widely within a given insect genus, indicating that the mountain ash scale and the ivy scale interchanged in his experiment may not have been identical species in *sensu strictu*. Rubtsov concludes that much of the host preference reactions of the scale are genetically fixed (217). That selection, rather than adaption was largely responsible for the results obtained in these various studies is evident from an examination of the mortality data presented, but attempts to accelerate the process of speciation constitute an interesting and neglected line of entomological research, regardless of their motives.

The influence of light and leaf color on host selection by Homoptera has been studied by Moericke (193) and Bonnemaïson (26). The results of these investigations can be harmoniously incorporated into a generalized theory on host selection that has been proposed by Nuorteva (201) as a result of his studies with leafhoppers, but which may apply to many other species. This author believes that (a) the insect is attracted to the plant by the overall color pattern, (b) olfactory stimuli bring it to rest on the proper plant tissue, (c) local color differences dictate the point of insertion of the mouth parts, (d) guidance of the stylets to the phloem is affected by a pH gradient, and (e) gustatory stimuli begin and maintain the rate of food intake.

SOIL FERTILITY AND NUTRITION OF PHYTOPHAGOUS INSECTS

Haseman (105) has called attention to the indirect influence of meteorological factors on insect development via climatic control of the physiological condition of the plant. This author has made suggestions concerning the limitation of nutrients that should be interpreted with caution by the practical farmer. Barber & Tauber (11, 12) have reported that the pea aphid exhibits lower reproductive rates on plants deficient in Ca, Mg, N, P, and K, but the health of the plant is reduced far beyond the margin of safety by these treatments. The greenbug, *Toxoptera graminum*, on the other hand declines in number following soil fertilization [Arant & Jones (3); Blickenstaff *et al.* (23)]. Fertilization of lemon with N retards development of red scale [Steyn (237)], and it has been suggested by Bobinskaia (24) that such effects on sucking insects are attributable as much to morphological changes in the deficient host that reduce physical attractiveness as they are a result of reduction in the food value of the cell sap. In the case of chewing insects, Smith & Northcott (234) report that wheat grown in nutrient solution rich in N is superior in nutritive value to N-deficient wheat for *Melanoplus mexicanus*.

In contrast to the above reports where variation of soil fertility has shown to effect the parasite, other groups have been unable to show important differences in growth or reproductive potential of certain homopterans [Hauschild (106); Barber & Tauber (10); Taylor and co-workers (245)]. Negative data has also been obtained with lepidopterans [Hocking (119); Douglas & Eckhardt (56)], and pests of stored grains [Eden (59)]. The various efforts along the lines described in this section would greatly benefit by the inclusion of analytical data which would show precisely the quantitative differences in as many of the essential nutrients as is possible to determine, as well as to show possible changes in the availability of these nutrients following the various fertilizer treatments.

Several contributions have been received since submission of the original manuscript. A personal communication from Dr. A. S. Crafts, California Agricultural Experiment Station at Davis, California, reveals that his laboratory has perfected an extrusion technique for the collection of milliliter quantities of phloem sap from cucurbits. Evidence favoring an ascorbic acid

requirement for *Bombyx mori* larvae has been obtained by Gamo & Seki (92a) who also have obtained indications of the synthesis of this vitamin from mannose in the pupal stage. Ishii & Hirano (126a) have extended their studies on the rice stem borer to the extent where a requirement for the 10 essential amino acids has been demonstrated. A recent review by Kuhn (155a) presents this worker's important experiments relating to the resistance of various species of potatoes to *L. decemlineata*. Pant & Fraenkel's studies on the vitamin requirements of symbiote-free Coleoptera have been extended to include *Oryzaephilus surinamensis* (207a). Finally, significant decreases in the oviposition rate of the mustard beetle, *Phaedon cochleariae*, have been observed following the feeding of watercress leaves deficient in N, P, K, or Fe [Allen & Selman (2a)].

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THE "LANGUAGE" AND ORIENTATION OF THE HONEY BEE¹

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We have been asked to report upon the latest results of our study of the "language" and orientation of bees. The knowledge we had up to 1950 has been presented in a series of lectures entitled "Bees" and given by the senior author on the occasion of an invitational tour of the United States. These lectures have been edited in book form by von Frisch (11), and a bibliography up to the year 1950 is to be found in this booklet. The present review is concerned primarily with developments since that time.

The "language" of the bees is not a verbal one. It depends on the senses of touch and smell. Their words are rhythmic movements and scents. Before going into details we should like to restate the means by which the bees communicate with each other.

A bee informs her hive companions of a new, rich food source (nectar, sugar solution, or pollen) found in the close vicinity of the hive by means of a round dance (see Fig. 2a). Those of the bees which are in a co-operative mood follow the returned bee closely during the dance on the honeycomb and learn that there is a profitable source of food. By the special form of dance, which gives no indication of direction, they are taught that it is to be found close to the hive. The specific scent of the visited plant sticking to the body of the dancer, to the pollen, or to the nectar tells them for which kind of flower to search.

If the food is farther away than 50 to 100 m., the round dance is replaced by the waggle dance (Fig. 2c). With its help the hive mates following the dancer are informed not only of the existence of a promising source of food and of its characteristic scent, they even learn at what distance and in which direction from the hive to seek it. The distance is taught by the measure (rhythm) of the dance [von Frisch (11)]. The direction is given in relation to the position of the sun. If the feeding place is to be found exactly in the same direction in which the sun lies at the time, the dancer indicates this on the vertical honeycomb of the hive by orientating the straight wagging of the dance vertically upwards. If the feeding place lies at an angle of 60° to the left of the sun, the straight part within the dance will also be carried out at an angle of 60° to the left of the vertical, and so forth. They transpose thus the angle to the sun, that has to be made while flying to the food source, into the field of the gravity when dancing on the honeycomb [von Frisch (11, Fig. 44, p. 77)]. The hive mates which have accompanied the dance fly out and will find the source of food according to the communications they have received.

¹ The survey of the literature pertaining to this review was completed in April, 1955.

The food gathering bees dance after each flight, as long as it is advantageous to draw further gatherers to the exploited feeding place. As soon as the food becomes scarce, they stop dancing, although they continue collecting, and from then on this worker group does not increase anymore. By these means the principle of relations between supply and demand is applied to the behaviour of bees.

Certain objections have been raised to our use of the word "language" to describe these means of communication [Révész (37)]. It is evident that the mental principles of communication between bees are quite different from those of the human language. In order to underline this difference we write the word "language" in quotation marks. There is, however, no doubt that the language of the bees is on a higher level than the means of communication among birds and mammals with the exception of man. Warning or attracting calls and mating songs among birds express only the animal's motivation. This can convey itself to other members of the species. The dances of bees, however, transmit the knowledge of significant facts [von Frisch (14, 17)].

THE INDICATION OF DISTANCE

With increasing distance between feeding place and hive the number of straight runs within the waggle dance decreases, the rhythm of the dance becomes slower. At the same time the number of abdominal waggles during the straight run increases. It cannot be determined from the beginning whether the number of straight runs in a given time, or the number of waggles, indicate the distances. The latter are so quick that one can only approximately estimate their number by direct observation. Relying on such data [von Frisch (9, p. 20)] Haldane & Spurway (20) suggest that the number of waggles is the principal means by which distance is conveyed. We think, however, that before coming to this conclusion we must count the mean number of waggles per distance as accurately as possible. For this reason, we have made slow-motion pictures of dances at varying distances from the feeding place. The evaluation of this work has not yet been completed.

Two important observations have been made which might answer the problem of how a bee is able to estimate the distance she has flown. If the bee has a head wind on its outward journey to the feeding place she indicates in her dance a greater distance than when there is no wind. Similarly she indicates a shorter distance than normal when she had the wind in her favour [von Frisch (10, p. 15)]. If she has to fly uphill on her way to the feeding place she will, when dancing in the hive convey a greater distance than when she flies the same length on the level. And if she has to fly downhill she will communicate a shorter distance still [Heran & Wanke (23)]. It seems, therefore, that the time or energy spent on the flight from hive to feeding place^{*} are very important, instead of the actual distance. Probably the main factor in the evaluation of distance is the energy spent on the flight; the experiments concerning this problem, however, are not yet finished.

* All given data on the dance concern the flight to the feeding place, none the flight homewards to the hive.

The following experiment shows how accurately the dancer's indication of distance is understood and followed by the other bees. Several bees individually numbered with coloured dots are, at a certain distance from the hive, fed with a sugar solution to which a scent, for example lavender oil, was added. Upon their return to the hive, the numbered gatherers dance, and during the dance the hive companions smell the lavender and search for this specific odour when flying out. On the direct line from the hive to the feeding place and even further away "scent plates" are placed at various distances. They emit the scent, but offer no food. The bees looking out for this odour at the indicated distance are attracted by the scent plates if they come near them. They fly around them and finally alight on them and are thereupon counted by an observer. In Figure 1 we show the results of two such experi-

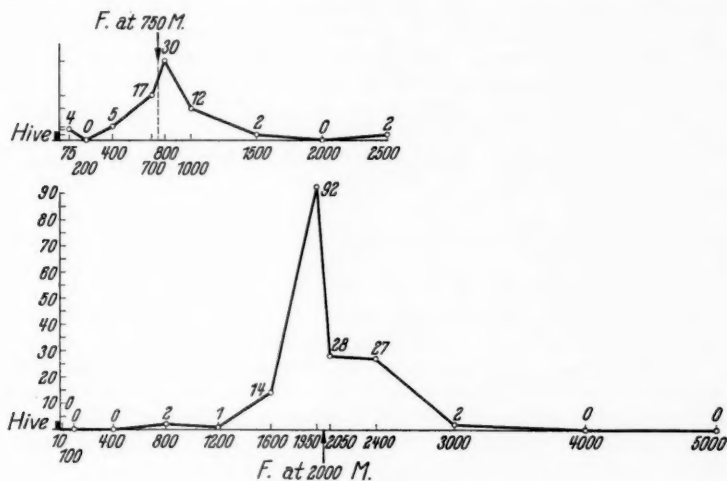


FIG. 1. Experiments concerning the reaction to the indication of distance. The distances of the scent plates from the hive are indicated on the abscissa in meters. The numbers on the points of the graph correspond with the number of bees which have come to each scent plate.

(Above) Trial of June 27, 1949. Distance between feeding place (F) and hive is 750 m. Duration of the experiment: 90 min.

(Below) Trial of July 20, 1952. Distance to feeding place is 2000 m. Duration of experiment: 3 hr.

ments. In the first trial the feeding place was at a distance of 750 m. and in the second at 2000 m. from the hive. The data given for the different points of the graph indicate how many of the searching bees came to each scent plate during the time of observation. The graphs show that the indication of distance given by the dancer is quite accurate and has been well understood by the other bees [von Frisch (13, 17)].

THE INDICATION OF DIRECTION

Normally the bees dance on the vertical honeycomb. In the dark hive they show the direction to be flown in order to indicate the feeding place to their companions by transposing the angle to the sun at which they flew from hive to food into the vertical plane. Sometimes, however, it happens that the returning bee dances outside the hive on the horizontal landing plank, in the daylight. In that case she points directly towards the goal during the straight wagging runs of the dance [von Frisch (11, Fig. 50, p. 87)]. This kind of communication of direction is the simpler one and more easily understood, as many insects possess a light-compass-reaction. They can register the position of the sun during their excursions or flights and keep thus a straight line of movement always maintaining the same angle to the sun. During her flight the bee also memorises the position of the sun and upon her return to the hive she maintains on the horizontal surface the same memorised angle to the sun, when performing the straight run of the dance. Thus she points directly towards the feeding place during the wagging dance.

Based on these observations we presumed that the dancing on a horizontal surface and in daylight was the more primitive and phylogenetically older form of indication of direction. It was, therefore, important to investigate the behaviour of other species of bees such as *Apis florea* Fabricius the dwarf honey bee. Its behaviour is comparatively primitive. The nest consists of one honeycomb only and possesses no nest cover. The honeycomb is built in the open air, suspended from a bush, the top of the comb being attached to a branch. The upper end of the honeycomb forms a broadened horizontal platform. The homecoming bees go immediately onto this platform where they convey, by a horizontal wagging dance in the same manner as our honey bees, what they found and whereabouts. They indicate the direction to be flown according to the position of the sun or the polarized light rays of the blue sky. If the honeycomb is turned around its horizontal or its vertical axis, the dancers walk always to the top in order to find a horizontal surface for dancing [Lindauer (unpublished observations)]. The hypothesis that the dancing on a horizontal surface is the original and more primitive means of communication among bees is considerably strengthened by these observations.

It is rather difficult to conceive how in their social evolution the bees have acquired the possibility of transposing their orientation to the sun into an orientation to gravity for the vertical dance in the dark hive. But some new observations show that among other insects such translations are also to be found, although biologically they appear to be pointless.

Vowles (41) working in a dark room on a horizontal plane forced ants into a motion of flight. As long as a lamp burnt the ants always maintained the same angle to the light source while in flight (light-compass-movement). When the light was turned out, and the surface put in a vertical position, the ant would run at approximately the same angle to the field of gravity as it formerly had taken to the light on the horizontal plane. Birukow (6) found

a similar behaviour in the dung beetle, *Geotrupes silvaticus* Panz., in *Coccinella septempunctata* Linn., and in *Melasoma populi* Linn. The conformity with the behaviour of the bees, however, is not complete. The ants do indeed maintain the same angle to gravity when on a vertical surface as they had kept to the light but with four possibilities of choice: they run at the same angle either upwards to the right or to the left, or downwards to the right or to the left. *Geotrupes* translates correctly not only the angle but even the direction to the light into the direction to gravity, with the difference that it identifies the downward direction with the direction to the sun and the upwards direction with the one leading away from the sun. The bees, on the other hand, direct their waggle dance to the zenith when they fly towards the sun and to the nadir when they fly away from the light. This latter behaviour can also be found in some other beetles (*Coccinella* and *Melasoma*). But when these ran, e.g., 20° to the left towards the light, they can in the dark and on a vertical plane run either 20° to the left or the right against the field of gravity [Teuckhoff, after Birukow (6)]. There is little doubt that with further research other insects will be found which transpose exactly in the same way as the bees. This, however, is not so very important. The essential fact is that there seems to exist a primary capacity of the nervous centers to transpose a given angle to the light into an action of the sense organ of gravity as soon as the optical stimulus ceases to act [Birukow (6)]. Rothschild (38) comes in a more philosophic way to the same conclusion. Therefore we need not suppose that on a conference of the workers union the bees agreed to adopt a certain key reaction in order to transpose the angle to the light into the angle to gravity. The fact that during their evolution they were able to make such a correct and unmistakable use of their primary faculty of transposition is miraculous enough.

We tried in vain to find an expression for "upwards" and for "downwards" in the "language" of the bees. We carried a hive to a deep valley setting it up in the middle of the steep slope. Then two feeding places were put out, both being in exactly the same direction from the hive and at the same distance, but one being on the valley floor, the other exactly above it on a bridge. The dancing bees were unable to inform their hive mates whether they meant them to fly to the upper or the lower feeding place. In another experiment the feeding place was exactly above the opening platform of the hive on a broadcasting tower. The dancers could not convey the vertical direction of flight to the other bees and performed round dances thus sending their companions out into the surrounding meadows instead of upwards to their own feeding place [von Frisch, Heran & Lindauer (18)].

"DIALECTS" IN THE BEES LANGUAGE

The bees belonging to the *carnica* race (*Apis mellifera* var. *carnica* Polm.) perform round dances (Fig. 2a) when the feeding place is closer than 50 to 100 m. from the hive. Baltzer and Tschumi found that Swiss bees execute another form of dancing, the sickle dance (Fig. 2b), when the feeding place is in the neighbourhood of the hive. Whereas the round dance of *A. m. carnica*

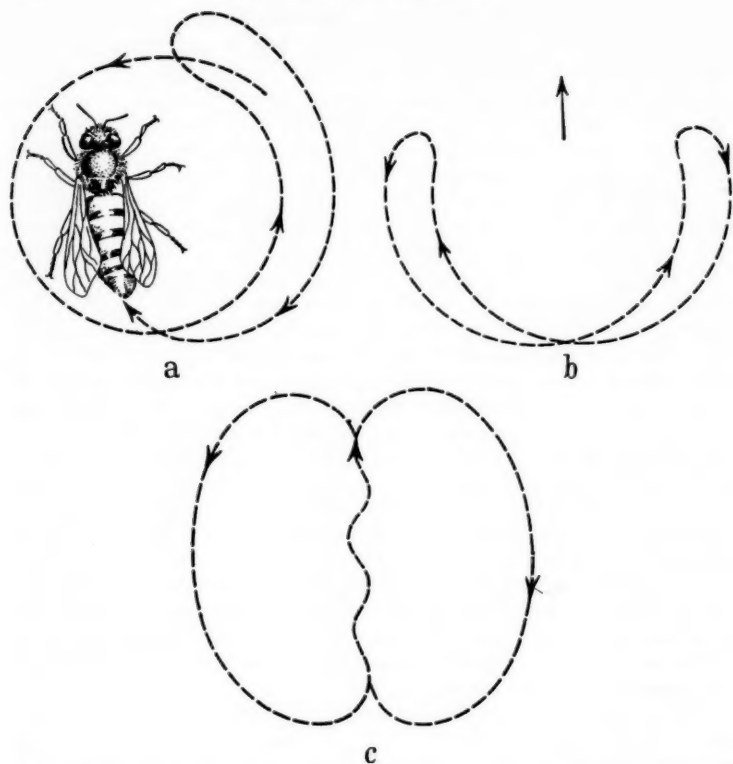


FIG. 2. The dance figures of the bees. (a) The round dance. (b) The sickle dance. (c) The waggle dance.

does not indicate the direction for feeding places in the immediate neighbourhood this sickle dance shows the direction to be flown. The axis of the danced semicircle (arrow in Fig. 2b) gives the appropriate direction to be followed, exactly in the same way as the straight part indicates this in the true waggle dance. According to Tschumi (40) the Swiss bees perform the sickle dance for as short a distance as 10 m. The same behaviour has been observed among Dutch bees by Hein (21).

This latter form of dancing seems to be a special character of the subspecies. In populations crossbred from the dark *A. m. carnica* and the yellow striped Italian bee (*Apis mellifera* var. *ligustica* Spin.) representatives of the dark race were to be found in the same hive with bees having the yellow abdominal segments of the Italian subspecies. Upon their return from a food source 10 m. distant the dark *carnica* bees performed mainly round dances,

whereas the yellow bees of the same population performed sickle dances [von Frisch (13)]. Further experiments with such cross-breeds have, however, shown that the form of the dance is not always coupled with the pigmentation of the dancer in this way. The pure Italian breed differs clearly however from the pure *carnica* colonies because of the large number of sickle dances performed at short feeding distances.³ Even the indication of distance reveals variations between these subspecies. At a given distance the Italian bees dance slower than the bees of *A. m. carnica* [Baltzer (2); von Frisch (13) and other unpublished experiments].

More important differences than those among the subspecies of our common honey bee have been found among the various species of *Apis* which Lindauer (unpublished data) was able to study on Ceylon.

Apis indica Fabr., closely related to *Apis mellifera* and often interpreted as one of its subspecies, indicates distance and direction in the same way as our honey bee but possesses the peculiarity that sickle dances indicating direction begin at a distance of 0.5 m. between the hive and source of food and that well directed waggle dances are performed as soon as the distance is above 2 m. The dance rhythm, for the indication of distance is slower than in *Apis mellifera*, although otherwise the graphs obtained from distance indication follow a very similar course. The dances of *A. dorsata* Fabr., the giant honey bee, differ from those of *A. m. carnica* only in that the direction-indicating waggle dances begin at as short a distance as 3 m. and that the dance rhythm for a given distance is slower. We have already mentioned that *A. florea* uses a horizontal surface for its dances. We would, however, like to add here that they perform round dances up to a distance of 5 m. between hive and food. As soon as the distance is longer they switch over to the waggle dance.

THE DANCES OF SWARMING BEES

The goal which the dancers indicate is normally a rich source of food. But during the search for a new nesting site exactly the same dances are carried out to convey information of good housing possibilities. Not only the daily bread but also favourable breeding conditions are highly important for the survival of a colony of bees.

A short time after a swarm has left the mother hive and formed a cluster in its neighbourhood, some bees may be seen dancing on the cluster. Now they are not food gatherers but scouts which have found a nesting site and indicate their discovery to the clustered, waiting colony. By close observation of these dances it is possible to predict the direction in which the swarm will finally fly and over what distance, sometimes several kilometers. On three occasions merely through the reading of the scout's dances we succeeded in finding, even before the colony moved to it, the new, hardly visible site.

At the beginning the scouts indicate several nesting sites to the cluster,

³ The colonies in which Tschumi and Hein observed the sickle dances were, as was discovered later, crossbred with Italian bees.

as they search everywhere at the same time. Each scout advertises his own discovery. Before the cluster moves into a new nest an agreement is, however, found, and the bees will fly to the best of all the proposed nesting sites. If on a small island or in a wide plain, with few or no natural sites, artificial nesting places are offered to a swarm, we are able to detect which qualities of a nesting site are favourably and which are adversely appraised by the scouting bees. Among other qualities the distance between nesting site and mother hive, the size of the nest cavity, its protection against wind, and its thermal insulation seem to be very important in their choice of the nesting place.

Those of the scouts which find an especially favourable site, dance much longer and more energetically than those which find a place of lesser value. In this way the attention of the clustered bees is mainly drawn to the best proposition. The bees that take part in the dances fly out and inspect the proposed nesting site. These, on their return to the cluster, also dance and make further propaganda for it.

We must add that those scouts that find an inferior site change their mind if other bees having found a better place dance very energetically near them. Attracted by these vigorous dancers they follow the latter and inspect the better place. Upon their return these also will dance in favour of the new goal.

We were also able to observe which of the bees became scouts. They are levied among the experienced field bees, which change their activity during the swarming time. These seasoned bees stop gathering when all the cells of the hive contain sealed brood, nectar, and pollen. Every apiarist knows that on such occasions the bees remain lazily and inactively in the hive and cluster in the form of a beard at the flight hole. He interprets his observation correctly as a sign for an approaching swarm emission. Under such circumstances, when there is no need for other activities some of the old bees take the initiative and reconnoiter new nesting sites. At this point the preparations for the swarming have begun. Thus the preliminary activities for swarming begin early, several days before the swarm leaves the hive [Lindauer (27, 28, 30)].

THE PERCEPTION OF THE SUN THROUGH A CLOUDED SKY

It is much more important for bees than for man to know always where the sun stands. This knowledge is indispensable for their own orientation and for the communication with their hive mates concerning feeding possibilities. In consequence they are much better equipped for such an accomplishment than we human beings.

They are even able to detect the position of the sun through the entirely covered sky. This can easily be shown if we put an experimental hive into a horizontal position and give the bees a view of the covered sky through a glass window. On the horizontal honeycomb the bees dance correctly. Their waggle lines point directly towards the goal, but only if they can see the point in the clouded sky behind which the sun is hidden. Their dance lacks orientation if they can not look in this direction, even if the other parts of the sky are visible.

This ability of detection is not attributable to the perception of infrared rays. To our great astonishment experiments with filters have shown that the ultraviolet rays between 3000 and 4000 Å are decisive for the bees' view of the sun when it is concealed behind the clouds [von Frisch (16); Heran (22)].

THE PERCEPTION OF POLARIZED LIGHT

If the sun is hidden behind a forest, a mountain, a house or even below the horizon, the bees are still able to orientate themselves and to convey correctly their findings under the condition that the sky, or only a small spot of it, is clear and blue. The light coming out of the blue sky is partially polarized, and its plane of vibration is in direct correlation with the position of the sun. The bee's eye is able to recognise the plane of vibration of polarized light and can in consequence detect the position of the unseen sun if a spot of blue sky is visible [see von Frisch (11, pp. 88-109)].

Autrum suggests that each ommatidium of the compound eye is able to analyse polarized light by its eight radially arranged sensory cells [see von Frisch (11, Fig. 55, p. 98)]. He strongly supported his hypothesis by carrying out electrophysiological experiments on eyes of bees and flies. The magnitude of the exposure potentials depends on the light intensity, if an ommatidium of a cut off head⁴ is exposed. The rotation of a polarizer put in front of the eye does not influence the magnitude of the potentials. In consequence the ommatidium does not possess a common polarizer. If this were the case maxima and minima would appear. Autrum found, however, that the exposure of an ommatidium with polarized light gives clearly higher potentials than the exposure with unpolarized light of the same intensity. This result corresponds very well with his hypothesis. In polarized light the intensity of the light becomes much stronger within the plane of its vibration when compared with unpolarized natural light of the same fundamental intensity. As a result of this fact the effective intensity becomes higher in those sensory cells of an ommatidium which are the most sensitive to the light of the given plane of vibration. It is irrelevant that the other sensory cells of the same ommatidium contribute a lower potential because the highest potential within the lot is decisive for the entire effect [Autrum & Stumpf (1)].

Optical research on the effect of polarization carried out with eyes of bees and flies have produced results which agree with these findings. Light arriving vertically on the ommatidium is not doubly refracted either by the corneal lens or by the crystalline cone. When the visual cells of an ommatidium are investigated in the direction of their axis, the rhabdomeres, however, show double refraction. Theoretically it is to be supposed that one of the two directions of vibration of double refracted light is suppressed in the visual

⁴ The head was put on a fine steel pin (indifferent electrode). The different electrode was a second pin stuck into the eye. The exposure of a single ommatidium was made possible by a source of light the angle of vision of which under the given conditions was considerably smaller for the eye than the angle of aperture of the ommatidium.

cell. The small size of the elements in question has not made it possible to prove this [Menzer & Stockhammer (31)]. It has been proved, however, that for flies (*Calliphora et al.*) the planes of vibration of the speedier rays are radially arranged and correspond thus with the position of the visual cells themselves.

Reactions to the direction of vibration of polarized light have, since their discovery among bees, been proved among many other Arthropoda: such as ants [Schifferer, see von Frisch (12, p. 220); Carthy (7); Vowles (41)], among flies [Autrum & Stumpf (1) for *Calliphora*; Wellington (45) for *Sarcophaga*; Stephens, Fingerman & Brown (39) for *Drosophila*], among beetles [Birukow (5) for *Geotrupes*], among larvae of Lepidoptera and Hymenoptera [Wellington, Sullivan & Green (46)], among larvae of Diptera [Baylor & Smith (3)], among crustaceans [Kerz (24) for *Eupagurus*], among Amphipoda [Pardi & Papi (33, 35, 36) for *Talitrus*], among several Cladocera [Baylor & Smith (3); Eckert (8)], among spiders for the wolf spider *Arctosa* [Papi (32)], among mites for *Hydrachna* [Baylor & Smith (3)] and finally in *Agalena* [Görner (unpublished data)] and in *Limulus* [Waterman (42, 43, 44)].

Some authors think that the reactions to polarized light, which they were able to observe, can be explained by a double refraction in the dioptric system. Such an explanation is however only possible if the effective light rays arrive obliquely (to the longitudinal axis of the ommatidium) on the eye [see Berger & Segal (4); Stephens, Fingerman & Brown (39); Baylor & Smith (3)]. It is possible that the reactions to polarized light of those Arthropoda which have been examined are not all attributable to the same type of function. For bees and flies, at least, we cannot agree with the above mentioned supposition.

The results obtained by Autrum & Stumpf, by Menzer & Stockhammer, and by Stockhammer indicate that there is a double refraction within the visual cells of each ommatidium. Furthermore the bees are able to analyse the direction of vibration of polarized light extremely well, even when the experimental conditions are such that we cannot assume the participation of obliquely arriving rays.

THE IMPORTANCE OF THE SUN AS A MEANS OF ORIENTATION

It is not difficult to conceive that the sun can be used as a compass when the bees take a short flight. By flying at a constant angle to the sun they are able to keep on a straight course. Furthermore we have seen that they use the angle to the sun in order to indicate the direction of a goal to their hive mates. Even this is easily comprehensible for short periods of activity.

During recent years we found that bees are capable of using the sun for orientation over periods of many hours during which the sun's position continually changes. They seem to be acquainted with the movement of the sun and to possess a well developed time sense which enables them to calculate the change of the solar position and to correct accordingly their direction of flight. The proof that they possess this faculty was first obtained by a dis-

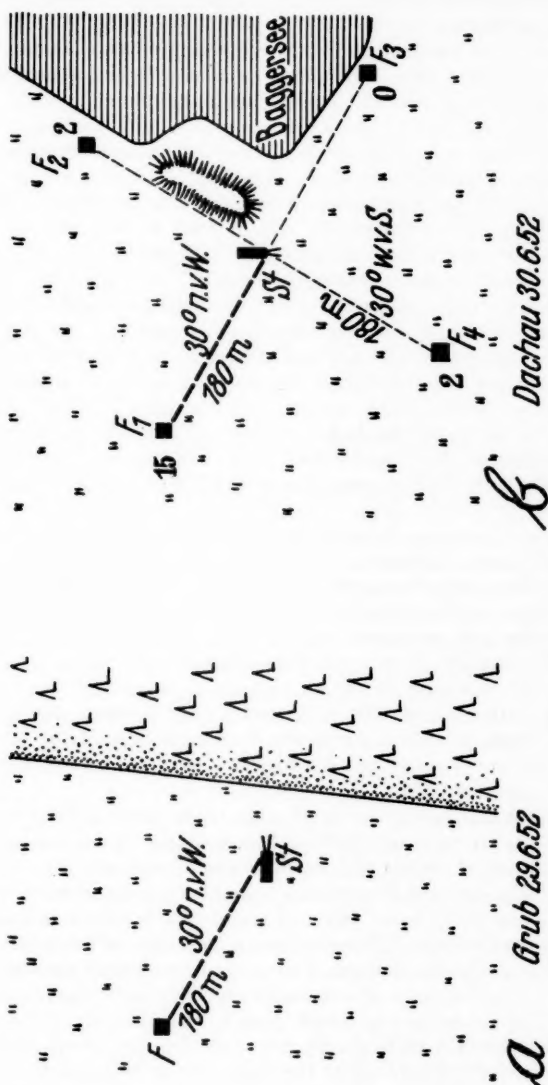


FIG. 3. Displacement test indicating ability of honey bee to allow for change in solar position. (a) Position of hive (St) and feeding place (F) on afternoon of June 29, 1952 near Grub. The marked bees were fed during this afternoon. (b) New position of hive and feeding places (F1 to F4) on the following morning (June 30) near Dachau. The numbers to the left of each feeding place indicate the numbers of previously marked bees collected at each feeding place this morning. Every arriving bee is immediately caught so that the other bees at the hive cannot be informed of the change in the situation.

placement test [von Frisch (12); von Frisch & Lindauer (19)]. As an example we show here the method and its results.

An experimental hive was brought into a region entirely unknown to the colony. On the afternoon of June 29, 1952 we opened the flight hole and fed some of the numbered and thus individually known bees until the evening. The feeding place was 180 m. from the hive in the direction 30° north of west (Fig. 3a). On the following morning the hive was taken to another entirely unknown landscape of different structure. The bees, therefore, found no familiar landmarks for their orientation during flight. The sun stood in the east, whereas during the preceding afternoon it had been in the west. Furthermore the hive had a different position. Thus the position of the flight hole could not help in the orientation of the bees. All around the hive at a distance of 180 m. we put up feeding plates. Notwithstanding the different landscape, the different position of hive and sun and in spite of the different time, the known bees, with few exceptions, flew to the feeding place 30° north of west which they had found and visited during the preceding afternoon. The other feeding places were almost completely ignored (Fig. 3b). In their search for the previously known feeding place in this unknown territory the bees can only have been helped by the sun. In order to use the sun as a means of orientation bees must be able to calculate the solar movements.

This capability is, however, shown in a much more surprising fashion by the following observation. Gathering bees do not dance for a longer time than 1 or 2 min. They start afterwards for a new flight. Some of the scouts become nonstop-dancers, when they find a nesting site for a preparing swarm, performing for hours their orientated waggle dance. During that time they slowly correct the direction of their dance according to the change of the sun's position. Even when they dance in a hive, set up in a closed room, so that they can see neither sun nor sky, they change their dance accordingly. They hereby demonstrate most impressively their knowledge of the solar position for every hour of the day and this even in a completely closed room [von Frisch (15); Lindauer (29)].

There is no doubt that among the Arthropoda the honey bees with their complicated instincts are on a very high rank of evolution. It is, however, remarkable that the use of the sun as a compass in correlation with a perfect time sense apparently does not necessitate a very high development of the mental faculties, because the same means of orientation is known to exist among crustaceans and spiders. *Talitrus saltator*, a crustacean which belongs to the Amphipoda, lives on the seashore. If by a storm or a human hand this animal is carried out of the region of moist sand which it inhabits and is put down on dry land, it makes his way hastily back to the shore. Even if it is carried many miles inland it takes exactly the same direction which would normally bring it from dry sand back to the moist sand of its habitat. This occurs only under the condition that it is given the opportunity of detecting the solar position, either directly or with the help of the polarized light from the blue sky. It finds this definite direction at every hour of the day even if

it has previously been kept for hours in a dark room [Pardi & Papi (33, 35, 36)]. The same means of orientation has been found in *Tylos latreillii*, a crustacean belonging to the Isopoda, which is also an inhabitant of the moist sand of beaches [Pardi (34)] and in the wolf spider *Arctosa perita*, which lives on the shores of the sea and lakes [Papi (32)]. It is surmisable that the sun serves also as a compass for the long distance migrations of insects such as butterflies. This method of orientation, is however, not only known among the Arthropoda but even among birds. Birds can use the sun in connection with the time of day as a guide for their migrations [Kramer (25, 26)].

THE RELATIVE IMPORTANCE OF LANDMARKS AND THE SOLAR COMPASS

It has been known for a long time that bees use conspicuous landmarks such as trees, rocks, houses, etc. for their orientation in the landscape. But as we have seen, they orientate themselves as well by the position of the sun and by polarized light. In order to find out which of the two, the terrestrial or the celestial clue, is the more important for the bee, we set them in competition in the following experiment.

We trained bees to find a feeding place 180 meters to the south of their hive. The arrangement was such that on their way from hive to food source the bees flew along the edge of a forest running from north to south. On the following day we transplanted the colony in a new unknown landscape with a similar edge of forest, this time running from east to west. The bees did not look for the feeding place in the trained direction to the south of the hive, but flew along the edge of the forest 180 m. to the west. The very conspicuous landmark won the competition over the solar compass. In another set of conditions, however, when the edge of the forest was 200 m. lateral to the course to be flown, the forest being at an angle of vision of 3 to 4°, after their transplantation the bees followed in the majority of cases the clue given by the celestial compass.

In the same way as an adjacent forest edge, a shore line or a road is a superior means of orientation when compared to solar orientation. When geographic characters are used it appears to be most important that the course to be flown follow a continuous and unbroken landmark. Correspondingly experiments have shown that a single high tree or an isolated cluster of bushes were not instrumental in deviating the bees from their accustomed direction of flight after a transplantation into a new landscape [von Frisch & Lindauer (19)].

Our method of investigation is time consuming but it gives us the possibility of judging the relative importance of the different means of orientation in bees.

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THE STABILITY OF SCIENTIFIC NAMES¹

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Stability, like virtue, is generally regarded as a desirable attribute, and most zoologists would agree that stability should be a prime objective of scientific nomenclature. However, the record of nearly 200 years under the Linnaean system of binomial nomenclature is characterized not so much by stability as by change. In entomology, especially, it seems that nearly every well-known name has been changed not once but several times. It is the purpose of the present paper to discuss the reasons for name changes and the recent actions of the International Commission on Zoological Nomenclature which, it is hoped, will lead to a more stable nomenclature.

ORIGIN OF THE BINOMIAL SYSTEM

Contrary to popular opinion, our present system of scientific nomenclature was not invented by Linnaeus but was merely formalized and applied universally by him. Actually, the system is a completely logical one and is used more or less consistently by all peoples in all languages. It employs a general term denoting the kind of organism (bear, fox, bee, fly) and usually a descriptive adjective (house fly, horse fly, etc.). The use of Latin (*Musca domestica*) was an accident of the times, this being the universal language of science in the eighteenth century. Thus the binomial system grew not out of the invention of one man but out of a common method of referring to things by the human species. Its usefulness is attested by the fact that it gained universal acceptance. Other systems might have been adopted and, in fact, were tried before and after the time of Linnaeus, but none of these has prevailed or even seriously threatened the binomial system. Perhaps this is because the present system is so good, but one should not overlook the possibility that in the future new and superior methods might be devised utilizing, for example, electronic business machines or library index systems.

For the present, however, it seems realistic to approach nomenclatural problems by improvement of the present system. What then are the strengths and weaknesses of the binomial system? First, as pointed out above, it is a logical system, employing, as a general rule, collective nouns and descriptive adjectives. Second, it is a consistent system, each animal (and plant) having a specific name (unfortunately, not always descriptive) and a generic or group name. Third, and most important to the subject of this paper, the binomial system provides stability plus flexibility! This was probably the greatest single factor in gaining general acceptance of the system, and curiously, this is the point that is so often misunderstood by nontaxonomists. The fact is that stability alone would be a straight-jacket to

¹ The survey of the literature pertaining to this review was completed in April, 1955.

taxonomists and would block progress. For example, the sugarbeet leafhopper was known universally as *Eutettix tenellus* (Baker) for nearly half a century. Then, in 1948 Oman (7) discovered that its true relationships lie with a group of leafhoppers in the Old World with the greatest concentration of closely allied species in the circum-Mediterranean Region. The change in name to *Circulifer tenellus* no doubt disturbed many people, but it opened new vistas, suggesting possibilities for biological control and further search for the virus of curlytop. In this instance the binomial system provided stability at the specific level and flexibility at the generic level.

Disadvantages of the system are (a) that names are no longer very descriptive because of the enormous number of species; (b) that Latin has ceased to be the universal language of science; and (c) the system has become so complicated that the average zoologist and even working taxonomists are reluctant to devote the time necessary to understand the Rules. The first two of these difficulties are more or less inevitable, but the third can be corrected to a certain extent by a simplified, up-to-date version of the Rules (now being prepared) and by streamlined procedures within the Commission (described below).

REASONS FOR NAME CHANGES

Probably the commonest reason for changing a scientific name is reclassification, as mentioned above in the discussion of the sugarbeet leafhopper. A nominal species (the name, not the population of actual organisms) may be transferred to another nominal genus or may be broken up if it is found to be composite. Another common reason for name changes is synonymy, the discovery that two or more nominal species actually refer to one and the same thing. Certainly these categories of change are legitimate and would be not only tolerated but encouraged by economic entomologists as advances in knowledge of the insects with which they are concerned. Muesebeck (11) gives other examples of this kind.

Some other types of name change are less palatable to nontaxonomists but are necessary consequences of the system. For example, the same name accidentally proposed for two different animals (homonyms) would cause confusion and therefore is not permitted. It has been argued that homonymous names in widely different groups such as insects and echinoderms would not be confusing, but a proposal to permit this was rejected because of difficulties in setting limits to the scheme (for example, a protozoan might occur in the gut of an insect with the same name, etc.). Another type of name change that causes great difficulty is attributable to "*nomina dubia*." Many dubious or unidentifiable names have accumulated during the past 200 years and remain to clutter up taxonomic literature. Each of these names (and there are thousands of them in the Insecta) is a potential source of trouble because of the possibility that someone may think that he can identify it and apply it as an earlier name for a well-known species. As a step in promoting stability

the International Commission at its Copenhagen meeting (C.D. 24)² set up a procedure for eliminating *nomina dubia*. All that is necessary is to propose the suppression of the name and send a notice to that effect to the International Commission for publication in the *Bulletin of Zoological Nomenclature*. The action comes into effect as of the date of publication but, in fairness to others, a challenge period of 10 years is permitted. If no question is raised in that time, the name is added to the Official Index of Rejected and Invalid names and cannot be used again.

Also there are name changes caused by technicalities in the Rules. Herein lies the greatest difficulty and misunderstanding. It is difficult for the non-taxonomist to understand why, for example, the name of a common insect should be changed merely because of an erroneous or even an accidental fixation 100 years ago of the type species of a genus. It is equally difficult for a person with no training in classical languages to appreciate why two generic names with apparently similar endings are of different gender so that one requires adjectival specific names in the neuter (*Belostoma flumineum*) and the other in the feminine (*Pentatoma rubida*).

Unfortunately, some technicalities appear to be inherent in any system or any set of rules. The alternative, of course, is to do without rules, and this has been seriously suggested by some taxonomists. One author, for example, proposed that the names used by the most recent monographer be followed. Unfortunately, such suggestions will not stand close scrutiny. Either they would end in complete anarchy, or monographers would have to follow rules which then would bring us back to our present situation.

ORIGIN AND DEVELOPMENT OF THE RULES

The present Rules are the result of over 200 years of trial and error, heated debate, and careful consideration. Linnaeus (6) proposed the first set of informal rules in his *Critica Botanica* (1737). Later landmarks include (9) the Stricklandian Code (1842), the Code (1, 2) of the American Ornithologists' Union (1886, 1908), the Entomological Code (3) of Banks & Caudell (1912) and others. At first the problems were relatively simple because only a few thousand names were involved. Now, with over a million names of animals the problem has become complex. Entomologists, in particular, will be concerned with nomenclatural rules and procedures for a long time to come because they must deal with 80 per cent of the names in the Animal Kingdom.

The growth of the Rules, or of codes of nomenclature as they are sometimes called, has been sporadic and has been marred by national and international controversies. In spite of the numerous difficulties, some of which still exist, zoologists should take pride in the fact that an international code

² In this review the designation "C.D." refers to the Copenhagen Decisions (see reference 4 of Literature Cited), and the number given is the exact page reference.

was agreed upon half a century before the United Nations Charter, and a truly International Commission has been working, however haltingly at times, for an equal period covering two world wars. This is no small achievement and should not be jettisoned hastily by those with a talent for oversimplification.

The International Rules grew out of various personal, national, and specialist group rules and gained acceptance through numerous compromises at the Fifth International Congress of Zoology in 1901. Immediately thereafter, questions arose as to their retroactive application to situations that took place in the 143 years prior to official Rules. Perhaps fortunately the Commission, through its Secretary, Charles W. Stiles, moved very slowly during this period. Nothing was done without virtual unanimity because there was no real legal force behind the Commission other than moral support from zoologists and from the International Congress and no real penalty for infringement except public censure. The effectiveness of the Rules was impaired in other ways during the first 34 years of their existence. For various reasons, no definitive text (the French text was the official one) was available, and, indeed, no up-to-date text has yet been prepared though one is promised in the near future. Meanwhile, a bewildering body of "case law" was built up out of "opinions" of the International Commission. These interpretations were necessary because the official text left so much unsaid.

By 1948 the situation had reached a climax. Only a few experts were thoroughly familiar with the Rules and Opinions, and nomenclature had fallen into disrepute in the eyes of general zoologists. A new secretary, Francis Hemming, had taken over the affairs of the Commission at its previous meeting (1935) before World War II. Thus the time was ripe for a thorough overhaul, and this was begun at the Paris meetings in 1948. Much needed reforms in procedure were adopted at that time and conspicuous gaps in the Rules were filled. Also the opinions were converted into the Rules, formalized by placement of names on Official Lists, or abolished.

But here again difficulties arose to mar the effectiveness of the action. Too much was done with too little time for careful consideration and without the usual formalities of notification of zoologists a year in advance. Then the Paris proceedings (8) were published in 760 pages at a price (\$32.50) that limited distribution to only a few of the largest libraries and research centers in the world. The resulting confusion and misunderstanding offset much of the good that was accomplished and lowered still further the opinion of scientists on the subject of nomenclature.

The most recent meeting of the Commission was held in conjunction with the 14th International Congress of Zoology at Copenhagen in 1953. Here, for the first time, an international colloquium on nomenclatural problems was held under the auspices of the Congress with assistance from UNESCO. The colloquium was truly international in representation and democratic in procedure. A sincere attempt was made to correct the mistakes of the past and solve the remaining problems so that a comprehensive revision of the Rules could be prepared for final approval at the London Congress which is scheduled for 1958. Such matters as family names, neotypes, and the names

of orders and higher groups were dealt with adequately for the first time. The Copenhagen Decisions (4) were published promptly, and in brief form and were generally available for the modest price of five shillings (75 \pounds). That the entire edition of this work was sold out in less than 18 months is evidence of the general interest in nomenclature.

RECENT CHANGES IN THE INTERNATIONAL RULES

The last complete edition of the Rules (5) in the English language was dated 1948 (changed only slightly from the 1926 edition). Since that time, as mentioned above, great strides have been made and in fact, a completely revised code is being prepared. Presumably a first draft will be published a year or so before the London Congress (1958) and, after careful consideration, will be debated, improved, and adopted in 1958. Meanwhile, the new provisions, although scattered and uncoordinated, are in effect and are to be followed. This is not unlike the situation when a driver enters a town and is not familiar with the details of its traffic laws. He may discover quickly and to his sorrow that ignorance is no excuse.

Since the last edition of the "Old Rules" nearly all additions are covered in the proceedings of the Paris (1948) and Copenhagen (1953) meetings. The distribution of these proceedings was limited so a summary of the important provisions is given below.

Infra-subspecific names (B.Z.N. 4:89)³.—At Paris (1948) official recognition was first given to names below the rank of subspecies. In order to do this it was first necessary to define the categories and forms of less than specific rank. The term "subspecies" was defined as a population (e.g., geographical, ecological) within a species that differs from any other such population within the same species. An "infra-subspecific form" was defined as any form of a species, other than a subspecies, e.g., seasonal, sexual, and transition forms, aberrations, and minority elements of all kinds within a species.

The problem of what to do with thousands of so-called "varieties," "forms," etc. already in the literature was dealt with by setting a time, December 31, 1950 to January 1, 1951, before which any name of less than specific rank shall be considered as a subspecific name, if in its original publication, the author either (a) clearly indicated that he regarded it as of subspecific rank, or (b) did not indicate its status (as defined above). Before 1951 the only names to be considered as infra-subspecific are those which an author, in the original publication, clearly indicated that he regarded as of infra-subspecific rank. Conversely after 1950, only those names which an author clearly indicates are of subspecific rank are to be so considered. All others, including those of doubtful status, are to be regarded as of infra-subspecific rank.

If subspecific names are reduced in rank they retain the original authorship and date whereas, if infra-subspecific names are raised they take the

³ In this review the designation "B.Z.N." refers to the *Bulletin of Zoological Nomenclature* which contains the proceedings of the Paris meetings (see reference 8 of Literature Cited). The numbers given refer to the volume and page of the exact reference.

new authorship and date. Subspecific names are written in trinomial form without intervening punctuation or explanation. Infra-subspecific names are written as qualified trinomials or quadrimomials, as follows: *X-us albus*, form *vernalis*.

Homonyms.—In the old Rules no distinction was made between so-called "primary" homonyms (combinations of generic and specific names which were identical when originally proposed) and "secondary" homonyms (combinations which became identical by the subsequent transfer of a nominal species). At Paris (B.Z.N. 4:105) it was ruled that the later published of two primary homonyms is to be replaced permanently, whenever discovered, but that secondary homonyms are to be replaced only if discovered while the condition of homonymy still exists. This relieved taxonomists of the necessity for endless searching of early literature for possible transfers (inadvertent or deliberate, correct or incorrect) of specific names from one genus to another. At Copenhagen (C.D. 82) a safeguard was adopted which permits a challenge of the renaming of secondary homonyms in the *Bulletin of Zoological Nomenclature*. Upon publication of such a protest the new name shall have availability only in the genus in which it was proposed whereas the original name shall be available in all other genera.

A list of equivalent spellings was adopted at Paris (B.Z.N. 4:162, 208, 243) to clarify what is meant by homonymous names; the alternate spellings given in each of the nine cases below are not to be considered as different for purposes of the Law of Homonymy: (a) the use of "ae," "oe," and "e"; (b) the use of "ei," "i," and "y"; (c) the use of "c" and "k"; (d) the aspiration or nonaspiration of a consonant; (e) the presence or absence of a "c" before a "t"; (f) the use of a single or double consonant; (g) the transcription of the semivowel or consonantal "i" as "y," "ei," "ej," or "ij"; (h) the use of "f" and "ph"; (i) the use of the particles "Mac" and "Mc" in names based on modern surnames that are otherwise identical. At Copenhagen (C.D. 78) this list was restricted in its application to names at the specific and infra-specific levels and the so-called "one-letter rule" was adopted for genera, stating that a generic name is not to be treated as a homonym of another such name if it differs in spelling by even one letter.

Family names.—At Paris (B.Z.N. 4:246) a start was made to clarify the very brief treatment of family names given in the original Rules but detailed consideration of the subject was left for Copenhagen (C.D. 32). The final provisions are too lengthy to reproduce here but cover the following points: all names above the genus and below the suborder (tribes, subfamilies, superfamilies, etc.) are to be referred to as Family-Group names; priority is to apply to such names but automatic provisions were set up for the maintenance of current usage wherever this would appear to be justifiable; all family-group names are coordinate, that is, for purposes of priority, a name published for a unit in any category in the Family-Group shall retain its original author and date even if it is later treated as belonging to a higher or lower category; a family group name becomes available from the date of its original publication regardless of the ending employed providing that it was clearly used to denote a supra-generic category and was not used merely

as a plural noun or adjective or in a vernacular sense; a family group name is to be based on the stem of the type genus; the name of any genus in the family may be selected as the type, not necessarily the oldest; the name of a family need not be changed if the name of the type genus is found to be a synonym; the name does need to be changed, however, if the name of the type genus is a homonym. In such cases, the new family group name is based on the valid name of the type genus, not upon some other genus which might be older. In other words, the family group name follows the name of its type genus.

Names of orders, classes, and phyla.—At Copenhagen (C.D. 38) names of the Order-Class Group and of the Phylum-Group were dealt with for the first time. It was agreed that the type method would be applied (by specialist committees reporting, it was hoped, by 1958) and that current usage should be the guiding rule rather than priority. No announcement has yet been made on this subject so it appears doubtful if the job can be completed as planned in time for the Linnaean bicentennial.

Emendations.—Changes in spelling of scientific names have been a constant source of confusion. The original rules (Art. 19) were vague on this subject and the Paris enactments were incomplete (B.Z.N. 4:68). At Copenhagen (C.D. 43) Article 19 was replaced by new provisions as follows: any breach of the mandatory provisions on the formation of names shall be corrected but the original authorship and date shall be retained; the following terms shall be applied to the several kinds of name changes:

- (a) Original Spelling. This is the spelling or spellings used in the original publication.
 - (i) Valid Original Spelling. It is the definitive spelling where there is no clear evidence in the original publication that an error has been committed, or that the name violates any of the mandatory provisions of Article 8 (generic names) or Article 14 (specific names). In case there are two or more original spellings without errors, the first subsequent user determines the valid spelling.
 - (ii) Invalid Original Spelling. A spelling that violated the mandatory provisions mentioned above or is based upon an inadvertent error (e.g., where an author states that he is proposing a name to honor Carolus Linnaeus but the name is printed *ninnæi*). This is to be corrected and to be treated as corrected wherever it occurs. It has no status in nomenclature, does not preoccupy for the purposes of the Law of Homonymy and is not available as a replacement name.
- (b) Subsequent Spelling. Any spelling published after the original publication.
 - (i) Emendation. Any subsequent change in spelling which the author makes clear is intentional. The correction of an Invalid Original Spelling is a *Valid Emendation*. It takes the place of an Invalid Original Spelling in every respect, including authorship and date. Any other intentional "correction" is an *Invalid Emendation*, and has status in nomenclature as a separate name

with its own author and date. It is a junior objective synonym of the name in its original form and is available as a replacement name. It preoccupies any later name of the same spelling, and its author and date are those of the invalidly proposed emendation.

- (ii) *Erroneous Subsequent Spelling.* Any change in the spelling of a previously published name other than an Emendation. These "errors" have no separate status in nomenclature, do not pre-occupy for the purpose of the Law of Homonymy, and are not available as replacement names.

Type specimens and localities.—At Paris (B.Z.N. 4:186) detailed provisions on type specimens were added to the Rules for the first time. Holotype, syntype, and lectotype were authorized and defined, and it was stated that they are to be regarded as the property of science. Also it was recommended that they be deposited in a museum, that they be marked with distinctive labels, and that full data be recorded from them.

The subject of neotypes was made a special item on the agenda at Copenhagen (C.D. 28) and, after extensive debate, was dealt with as follows: A neotype is a single specimen designated to replace a holotype or lectotype that is believed to have been lost or destroyed. To prevent the casual or irresponsible designation of neotypes it was stated that neotypes are to be designated only in exceptional circumstances when they are desirable in the interests of stability or when they are essential in solving a confused taxonomic problem. Neotypes are not to be established for names which are not in general use. In order to establish a neotype an author must (a) publish his designation and send a note to the effect to the Secretary to the Commission for publication in the *Bulletin of Zoological Nomenclature*; (b) state his reason for believing all the type material is lost; (c) see that the neotype becomes the property of a recognized scientific or educational institution which maintains a research zoological collection with proper facilities for preserving types and which gives research scientists access to its collection; (d) provide evidence that the neotype is consistent with what is known of the original type material and with action taken by previous revisers; (e) give a precise description of the neotype or a bibliographic reference to such description or figure, with a definite statement that the neotype is consistent with that description or figure; (f) show that the neotype came from as near as possible to the locality, host, or horizon from which the original holotype or lectotype was located. If part of the original type material is rediscovered after a neotype has been designated, any author, acting as first reviser, may decide whether stability and universality of nomenclature will be better promoted by retaining the neotype and suppressing the original type material or by the contrary procedure. Neotypes designated prior to the Copenhagen Congress have no official status but can acquire such status through the provisions specified above (10, p. 239).

Provision was made at Copenhagen (C.D. 26) for the restriction of type localities when the original locality cited is lacking in sufficient precision and no further information is obtainable from the label affixed to the holotype or lectotype. In such cases, or where the original author did not cite a locality

or cited an erroneous locality, it shall be open to a first reviser to specify a restricted area and it shall be open to any later taxonomist further to restrict the locality.

Miscellaneous provisions.—Literally hundreds of small points were dealt with at Paris and Copenhagen and will be incorporated in the new Rules. Some of these were merely drafting amendments but others included new terminology and small but far reaching points in nomenclatural procedure. Of the former, the term "taxon" (C.D. 20) was adopted as a convenient way to refer to a "taxonomic unit" at any level and "nominate" (C.D. 21) was proposed in place of "nominotypical" for the subdivision of a taxon which bears the same name (the earliest) and the same type. Also "binomen" and "trinomen" were adopted to denote the binomial and trinomial combinations of species and subspecies (C.D. 20).

Small but far reaching provisions include: a precise definition of what constitutes "publication" (C.D. 60); provisions for determining the gender of generic names (C.D. 49); a rule that diacritic marks are not to be used in scientific names (C.D. 57), cases already in the literature to be changed in accordance with instructions to be provided; provision that new combinations are to be recognized (C.D. 59) and that, in transferring a nominal species from one nominal genus to another it is not necessary that the author actually cite the specific name in immediate juxtaposition with the name of the genus to create the combination; a provision to substitute "page precedence" for the rule of the "first reviser," passed at Paris (B.Z.N. 4:330), was reversed at Copenhagen (C.D. 66).

STABILITY

The most important subject that was dealt with at Copenhagen was the "means for promoting stability and universality in Zoological Nomenclature." This was a special order of business and became the underlying theme for the entire meeting. It is at once the oldest question in zoological nomenclature and the most recent. Having laid a certain amount of background and summarized the recent changes in the Rules, I now propose to return to this issue with the hope that its present status can be explained and detailed procedures can be outlined for stabilizing names in the future.

The basic issue in any consideration of stability in nomenclature is Priority versus Conservation. This question has caused heated debate for over 100 years and is raised whenever a well-known name is suppressed by a little known name of earlier date. The advocates of strict priority claim that it is in keeping with the scientific spirit to recognize the names of works of earliest date. They feel that the system is completely impersonal and automatic (except in rare instances where two works were published on the same date). Name changes, it is felt, are frequent in the early stages of a science but are ironed out sooner or later and cause inconvenience for only a comparatively few years.

On the other hand, the proponents of the Principle of Conservation feel that rules should be completely subservient to the people who make them, that they are relatively recent anyway, and that they should be set aside

whenever they threaten the stability of a well-known name. This position seemed so reasonable that it was implemented by the International Congress at Monaco in 1913. At that time, the Commission was granted Plenary Powers to set aside the Rules when, in its judgment, their strict application would "clearly result in greater confusion than uniformity." This was a great step in the direction of stability, providing machinery for the conservation of well-known names. Unfortunately, the machinery did not work very well. Relatively few names were saved in the 35 years up to the time of the Paris meeting in 1948, and the Plenary Powers procedure required as much as 20 years in some cases. Nevertheless, the principle was established and certain names such as *Musca domestica* Linnaeus, *Cimex lectularius* Linnaeus, etc. were placed on the "Official List." Such names were comparable to the *Nomina Conservanda* of the botanists. Many more names were considered at Paris and in the following years, and new lists were approved for generic names, family names, names and works that have been rejected, etc. The Commission has announced that a volume is being prepared to bring all such names on Official Lists together. After publication of the basic lists it should be possible to keep them up to date by issuing supplements.

THE SITUATION AT COPENHAGEN

When the colloquium convened at Copenhagen the situation was essentially as follows. Adherents of strict priority still felt that their method was the only one likely to achieve stability in the long run. On the other hand the advocates of continuity of names regardless of priority were not satisfied even with the streamlined Paris procedures for invoking the Plenary Powers. As they put it so eloquently and to the accompaniment of much applause and stamping of feet from the audience, they wanted to do away with grave-digging for old, long forgotten names. They wished to use current names regardless of priority so as to be free to proceed with more important matters. This feeling was so strong and so fiercely defended by blocks of persons segregated, unfortunately, along nationalist lines, that it dominated the colloquium and the Section on Nomenclature of the Congress. Under these circumstances a new Principle of Conservation was written into the Rules. No agreement was reached on any of the several proposals for implementing this principle. However, the following general statement was adopted.

The Rules should include a provision limiting the application of the Law of Priority in such a way as to preserve any well-known name which had been in general use for a considerable period from being sunk as a junior synonym of some much older name which had not been used more than a small number of times in a specified recent period of considerable length, this provision to contain also the best safeguards that could be devised to prevent the abuse of the proposed provision. . . .

The Commission was charged with the duty of preparing a definite rule along these lines and such a provision will undoubtedly form a part of the revised code.

The Principle of Conservation, although vague at the moment, is of the utmost importance to every entomologist. It is now clear (a) that well-established names do not need to be changed for nomenclatural reasons;

(b) that, when a well-known and commonly used name is found to be invalid, the fact should be reported to the Commission at once for possible action under the Plenary Powers; (c) that names which fit the criteria of usage (to be spelled out in the new code) are not to be changed but are to be conserved by notification in the *Bulletin of Zoological Nomenclature* with a two-year period allowed for protests before the action becomes final.

AUTOMATIC PROCEDURE

Prior to the Copenhagen meeting it was necessary to submit all questions to the Commission and wait for months or, in the past, for years before an answer was forthcoming. Such an arrangement was unsatisfactory for two reasons. First the delay in coming to a decision created confusion in general usage. But far more important than this was the effect on taxonomists. Generally, nomenclatural problems are a nuisance to the working taxonomist. He is obliged to deal with them in the course of his studies as a means to an end but he does not and should not look with favor on the prospect of holding up publication of his research while awaiting nomenclatural decisions. Therefore, most taxonomists have been reluctant to go through the laborious and time consuming process of applying for conservation of a name. Instead they have simply followed the rules. The result has been good in a way—because the Commission has not been overloaded and progress has not been hindered—but bad in another way—because so many well known names have been changed.

At Copenhagen a procedure was adopted which may solve the dilemma. It was decided that taxonomists should be permitted to take definitive action on questions of nomenclature at the time when the work is being done. Such actions are then to be published as notices in the *Bulletin of Zoological Nomenclature* and are subject to challenge by anyone during a specified period (two years for names under the Principle of Conservation, 10 years for *nomina dubia*, etc.). If no challenge has been made during the specified period, the action becomes final. If, on the other hand, questions are raised, the Commission considers all evidence and comes to a final decision. Thus an automatic procedure is established which, it is hoped will enable the working taxonomist to retain well known names and at the same time save the Commission from being deluged with routine applications, only a few of which may prove to be really controversial. How well this procedure will work remains to be seen, but one thing is already clear. It will not work unless taxonomists use it, and this will require far greater understanding and participation in nomenclatural matters than exists at present.

THE PROPOSED INTERNATIONAL ASSOCIATION FOR ZOOLOGICAL NOMENCLATURE

It is no accident that taxonomists and all persons who use scientific names are poorly informed on nomenclatural matters. As mentioned earlier in this discussion, the subject matter is complex, the proceedings are verbose and abstruse, and the *Bulletin of Zoological Nomenclature* is priced beyond the limits of all but a few of the largest libraries of the world. This situation

is, of course, intolerable and could, in itself, destroy all of the painstaking labor of 200 years. In an attempt to solve this basic problem the Copenhagen Congress appointed an Interim Committee to explore methods for broadening the financial support of the hopelessly overworked Commission and for disseminating the Commission's publications as widely as possible. The key point in the "Copenhagen Plan," as proposed by the Interim Committee, is an international association for the support of nomenclature to be activated at the London Congress in 1958. It is felt that only in this way can the financial burden of this essential activity be spread equitably. Instead of sending a high priced *Bulletin* to a few libraries, it is proposed to send a cheap *Bulletin* to thousands of individual taxonomists, the unit cost being reduced by distribution of large numbers of copies and by more economical methods of printing and editing. Thus every working taxonomist will be able to have a desk copy of the *Bulletin* and will become an active participant in the effort to achieve stability in names.

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SOME ASPECTS OF GEOGRAPHIC VARIATION IN INSECTS¹

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To survey the whole subject of geographic variation in insects would, in effect, mean reviewing most of population systematics. One would also have to consider much that is usually thought of as genetics, cytology, physiology, and ecology. This is because the evolutionary synthesis of which population systematics is part has so permeated these fields that cytologists may now be found studying the geographic variation of grasshoppers, and geneticists the effects of isolation in producing subspecies. Population systematics owes much of its content to the study of variation in insects, including their geographic variation. The best known of all animal populations are those of the fruit flies of the genus *Drosophila*; indeed, more is probably known about the biology of these Diptera, including the genetics of their geographic variation, than about that of any other group of organisms.

The recent publication of Mayr, Linsley & Usinger's *Methods and Principles of Systematic Zoology* (26) fortunately brings within reach of everyone an authoritative, comprehensive, and well-documented account of population systematics, in which geographic variation is discussed and illustrated by many examples in insects. This work and the evolutionary "classics" cited below will give the reader a broad survey of geographic variation that could not be compressed within the limits of this review. Only certain phases of geographic variation, chiefly in insect populations, chosen principally because they relate to common problems of classification or nomenclature, will be treated here. The author wishes first, however, to point out how widely the study of geographic variation has spread, and then to comment upon certain theoretical aspects of population systematics.

The study of geographic variation in fields other than taxonomy.—In looking over the entries in *Biological Abstracts* for 1945 to 1955, one is struck not only by the large number of papers treating some phase of geographic variation, but also by their diversity. Among more than 400 listed, almost one-half dealt wholly or in part with insects. The titles were found somewhat arbitrarily classified under the following heads (arranged in approximate order of included number): Genetics, Evolution, Cytology, Ecology, Taxonomy and Nomenclature, Systematic Entomology, Biometry, Economic Entomology, and Animal Behavior.

It is obvious that whereas geographic variation was once of interest principally to taxonomists, zoogeographers, and students of evolution, and

¹ The survey of the literature pertaining to this review was completed in July, 1955.

related almost wholly to morphology, it is now a subject of investigation in most of the major fields of biology and applies to any aspect of organisms that varies geographically. The variations may be in incidence of genes or gene arrangements, in rate of embryological development, in habitat selection, in choice of food, or in mating behavior, to mention actual examples. Viewpoint alone determines whether variation in such things is a systematic phenomenon or comes under the head of genetics, physiology, ecology, or behavior. Differences of this nature can, of course, be used in classification, although they are seldom important as taxonomic characters because they are known in so few species; their real significance is for systematic theory.

Theory and practice in the "new systematics."—The evolutionary synthesis of which population systematics is an important element first gained wide attention with the appearance, between 1937 and 1942, of four books which no biologist, whatever his interests, can afford not to know. These, classics of the field are Dobzhansky's *Genetics and the Origin of Species* (8), a volume of essays edited by Huxley entitled *The New Systematics* (20), Mayr's *Systematics and the Origin of Species* (24), and Huxley's *Evolution: The Modern Synthesis* (21).

Among later contributions only a few of the more important will be mentioned. In 1949 Emerson reviewed the ecological aspects of evolution and population systematics, and summarized a vast amount of the relevant literature [in Allee *et al.* (9), Sect. V]. That same year there appeared a symposium edited by Jepsen *et al.* (23) in which geneticists, paleontologists, and systematists summarized the contributions of those fields to the study of evolution. Two recent botanical books containing ideas important for population systematics may be mentioned here: Anderson's 1949 study of introgressive hybridization (1), and Stebbins' large 1950 work (37) on variation and evolution in plants. To our list of basic references we should also add the 1953 textbook by Mayr *et al.* (26) already cited, and the 1954 symposium entitled *Evolution as a Process*, edited by Huxley *et al.* (22) and noteworthy for the excellence of the included papers. Considered together, the books named present a full and many-sided account of modern evolutionary theory, with which population systematics is inextricably interwoven. They also contain references to nearly all important prior contributions.

In all these treatments of the "new systematics" demonstrated fact is of course distinguished from hypothesis and interpretation; but so neatly are they fitted together in composing the completed picture that one forgets it is a mosaic. In fact the result is actually not so much a mosaic as a blending of fact and hypothesis and of contributions from many fields; in it one cannot clearly tell where population systematics passes into population genetics or population dynamics or evolutionary theory. This is, of course, exactly what is meant by the evolutionary synthesis. Dissolving boundaries and intermingling ideas have unified and stimulated all phases of biology. There is, however, another side to remember: distinctions have also become blurred, and confusion is often the result.

Mayr *et al.* (26) have given us a useful shorthand notation for distinguish-

ing three levels of taxonomy: *alpha*, in which species are described, *beta*, in which species and subspecies are arranged in a hierarchy of categories, and *gamma*, or population systematics, in which infraspecific variation and evolution are studied. Entomological taxonomy is still a mixture of *alpha* and *beta* taxonomy and likely to remain so for a long time; in it the *gamma* level has been reached in only a few restricted groups, among which *Drosophila* soars to a lonely peak.

Among *beta* taxonomists who aspire to use *gamma* concepts and criteria in their studies, one finds a general failure to distinguish between the two aspects of population systematics, the actual study of infraspecific variation, and its theoretic and evolutionary interpretation. For convenience in this article these will be referred to respectively as *gamma*-1 and *gamma*-2. The first may be carried out independently of the second, though both are combined in many studies. The point to be made here is that *gamma*-1 taxonomy can be applied to any population of which the available hypodigm [Simpson (33)] includes adequate specimen-series from enough places in the range. The step from *gamma*-1 to *gamma*-2 taxonomy may, however, be a very long one, which should be made on the evidence, not by analogy only or by appeal to theory or authority. We shall return to this matter farther on.

In *gamma*-2 taxonomy, or evolutionary systematics, it is theory which is sought, not the explanation of particular instances. Investigations are made chiefly to test hypothesis and develop principle. Extrapolation is necessarily extensive. Most of what we know about the genetics and dynamics of real populations has come from observation and experiment on a very small number of kinds of animals and somewhat more numerous plants, a very minute sample upon which to generalize. Even more abstract is the theoretic basis of much of population dynamics, the mathematical analysis of imaginary populations with assumed attributes considered as existing under postulated conditions. Upon this small base of fact and reasoning rests the edifice of *gamma*-2 theory, well propped on all sides, it is true, by data from cytology, ecology, zoogeography, physiology, and *gamma*-1 taxonomy.

Evolutionary systematics emphasizes the principle that any given evolutionary result must be the product of complex interplay between many and varied factors. (A corollary is that every population and every taxonomic situation must be essentially unique.) It is capable of accommodating all conceivable evolutionary and taxonomic situations. In this hospitable atmosphere hypothesis flourishes, stimulating research designed to test it.

Unfortunately direct test of hypothesis is impossible in some areas, notably those in which time and historic event play important roles. A case in point is Mayr's (24) well-reasoned presentation of the principles of population systematics in which the basic ingredients are geographic variation, allopatry and sympatry, different kinds and degrees of isolation, and, of course, the properties and relations of populations. The conceptual scheme thus given form is now generally accepted as essentially true, even though in large part it is not susceptible of "proof" other than that it is internally consistent, accords with known facts, makes "good sense," and is useful in

thinking. One sometimes wonders, however, whether there is not implicit in it the assumption that in theory all varieties of systematic situations can be foreseen and given a place, so that each example encountered in nature need only be assigned to the category prearranged for it.

There seems to me some danger that, in practicing the "new systematics," theory will often be prematurely accepted as fact and misapplied. Population systematics is now in fashion, and everyone feels obliged to speak its language and "explain" in its terms. There is a vogue for the latest in theory, as witness the rapid rise and decline of genetic drift as an explanation of all change in small isolated populations, and the faddish way in which new terms and new ideas are made into "catch phrases" to be dragged into every discussion. More serious, we begin to encounter in taxonomic papers the facile, patterned "explanation" based on analogy and bolstered by authority. Without its authors being able to prevent it, the book by Mayr *et al.* (26) is by way of becoming the bible of taxonomy, to be quoted in support of one or another interpretation. If it be true that the reviser of a difficult genus had best make his own classification from his own observations before looking at what his predecessors have done, is it not equally desirable that the student of populations first form his conclusions and then see how they fit with theory?

And this brings us again to the question of how far the taxonomist who must work only with specimens can use population systematics in his studies. Is he limited to the *gamma*-1 level, or may he legitimately apply *gamma*-2 theory in interpreting his results? The answer must be: "That depends"—on the nature and amount of available data, on relevant collateral information, and most of all on the good judgment of the investigator, upon which everything in the end does depend.

THE STUDY OF GEOGRAPHIC VARIATION IN INSECT POPULATIONS

Most of those who read this will presumably be neither geneticists nor biometricians, but entomologists who encounter geographic variation in the insects they study and have to decide what it means and what to do about it. From their standpoint, and the author's, it is a practical problem, however much of theoretical interest it may also have. As such it may best be approached by considering actual instances, how they have been treated at the *gamma*-1 level, and some of their possible *gamma*-2 interpretations. The examples have been selected to illustrate situations rather than to represent a wide variety of insects.

Methods of study.—Analysis of samples is our only means of predicting the properties of "natural" populations, which are themselves forever inaccessible to study. Biometric analysis of series of specimens is standard practice in vertebrate taxonomy and is increasingly common in entomology. Manuals describing the applications of statistics to zoology are available at all levels from elementary to advanced [cf. Cazier & Bacon (4); Simpson & Roe (34); Snedecor (36)]. The method of discriminant analysis, based on multiple characters, is a useful but laborious tool for determining relative

degree of difference between several population samples [Fisher (10)]. Clark (5) has recently described an easier and taxonomically more practical way of obtaining comparable estimates. Hubbs & Perlmutter (19) show how means and variance of a series of samples can be simply graphed, and Dice (7) has discussed the role of quantitative methods in systematics.

In quantitative treatments populations are described in terms of the incidence of alternative characters (discontinuous variation) or in mean and variance of mensurable characters (continuous variation). But though all individual differences can in theory be reduced to number, in taxonomic practice there is a middle ground between the strictly quantitative and the purely subjective. Among complicated structures, of which insect genitalia are excellent examples, there are often differences of form which are easier to appreciate than to analyze into their components. Even these can be mathematically treated by assignment to classes, but this is seldom done in insect taxonomy. Instead, because of limitations upon time and effort, analysis of series by "inspection and estimate" is the rule in entomology. Such analysis is, of course, no substitute for quantitative mathematical treatment, but it can cover far more ground, and in the hands of an experienced and judicious taxonomist who thoroughly knows his group it can yield results which subsequent biometric studies serve only to confirm. Indeed, perhaps the commonest application of statistics to taxonomy is in testing a conclusion already reached through "inspection and estimate." Unfortunately not all taxonomists have the specified qualifications; but on the other hand, not all those who flourish statistics understand their use, and many an earnest student has mistaken the properties of numbers for those of populations.

Kinds of geographic variation.—Geographically correlated differences between populations can be classified either according to their spatial patterns and relations (*gamma*-1), or their biological significance (*gamma*-2). The accompanying over-simplified diagram (see Fig. 1) shows how the two

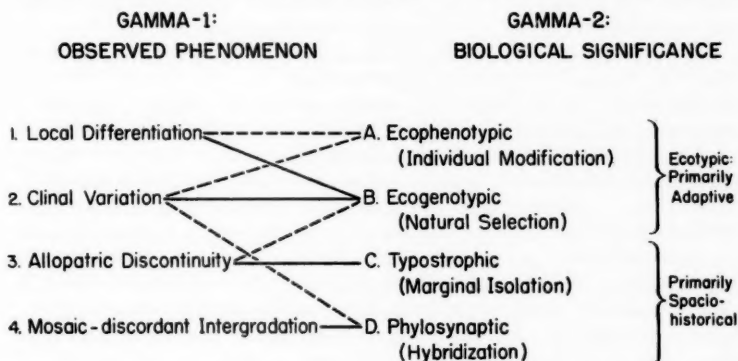


FIG. 1. Geographic variation in populations.

classifications are related and that the categories of each are heterogeneous in terms of those of the other. Solid lines indicate the more usual or more important relations, dashes others which may also exist. It should be noted that in the first column "clinal variation" has to do with changes in single characters, the other categories with multicharacter variation and populations. Some of the terms and concepts used require brief comment.

The term "cline," aptly coined by Huxley for the gradual and continuous change of a character from one region to another, has come into general use in that sense. However, its meaning is now often stretched to include concurrent clinal change in several characters and still further to mean the series of contiguous populations through which a clinal gradient extends (population-cline). Huxley himself called attention to the "stepped cline," in which the gradient of change is much steeper in some places than in others. It is best to use "cline" only in reference to single-character gradients, of which any number may occur in a population or extend through contiguous populations, running together or independently.

The regional change in populations that consists of the sum of all clinal and other geographic variation can be called "gradation"; and a corresponding multiple-character transition between populations is "intergradation," a term which may be descriptively qualified, for example as clinal, complex, mosaic-discordant, or localized. "Mosaic-discordant intergradation" is here defined as the occurrence, usually between two distinct and rather stable larger populations, of numerous small local populations that differ from one another in the incidence, degree of intermediacy of, and association between the taxonomic characters by which the bounding populations are distinguished. Such intergradation may be differentiated from that which is merely a coincidence of clinal steps by its heterogeneity, its irregularly mosaic pattern, the frequent discordance of its character-combinations, and the occasional novel features that arise in relation to it. The stepped clinal type of intergradation normally coincides with a pronounced environmental change, while in the mosaic-discordant type there is no such necessary relation.

"Allopatric" and "sympatric" are, respectively, the terms used by Mayr to distinguish populations with separate ranges from those with overlapping ranges; the situations they describe are unlike in their evolutionary and systematic implications. "Ecotypic variation" is primarily adaptive; it may be the result of individual response to environment ("ecophenotypic"), of genetic modification of a population by natural selection ("ecogenotypic"), or be a combination of both. "Typostrophic variation" is a term recently introduced by Mayr (24) to designate the large and rapid changes that may occur in isolated marginal populations, even those of considerable size. *Phylosynapsis* (from the Greek, *race*+*junction*) is a word here coined to mean the coming into contact of previously separated but not genetically isolated populations, and the resultant hybridization. Such contacts are likely to produce mosaic-discordant intergradation, but this depends on the kind and degree of reproductive isolation and other circumstances. The re-

sult may be anything from gradual clinal gradation at one extreme, to introgressive hybridization at localized centers between sympatric species which elsewhere show complete reproductive isolation.

EXAMPLES OF GEOGRAPHIC VARIATION IN INSECTS

The following examples, nearly all from Orthoptera, were chosen only in part because they are good illustrations of the varieties of geographic variation; others equally apropos could have been found in the taxonomic literature of most insect orders. They were selected principally because of first hand knowledge, the author having traced out the variations in the field and studied samples in the museum. In this section only the observed facts are described, their interpretation being reserved for the next. Some of the examples are presented in much more detail than others, either because unpublished data are involved or because they are used to illustrate more than one of the topics discussed in the next section.

The micropopulations of Melanoplus puer.—This small, flightless Florida grasshopper, like many other insects of the southeastern Coastal Plain, shows great diversity in a very small range. Its variation was described in 1932 (15); subsequent intensive field work has added detail to the sketch which was then given without altering its outline. *Melanoplus puer* ranges from Alachua County in the north to the southern tip of Florida and occupies the full breadth of the state south of the level of Eau Gallie on the east coast. East of the St. Johns River it is entirely replaced by the geminate but very distinct *Melanoplus adelogyrus*; both species are wholly absent from the Ocala National Forest, an "uninhabited island" in the midst of the state, isolated by the Oklawaha and St. Johns rivers and a belt of ecological barriers extending between them. South of Lake Okeechobee *M. puer* is probably nearly or quite absent from most of the Everglades, but it extends on the west coast south to Naples and on the eastern rim to Homestead.

Of general occurrence in the pine flatwoods of south Florida, *M. puer* is northward increasingly restricted to dry, open oak-pine woods on sandy soils. In much of peninsular Florida this type of habitat is interrupted by swamp, wet prairie, flatwoods, and "hammock" to such a degree that it may be likened to an archipelago, containing a multitude of "islands" (often very sharply defined) that range in area from many square miles to only a few acres. The result is to subdivide the *puer* population into a corresponding set of larger and smaller local populations, ecologically isolated from one another in varying degree. In the central part of the *puer* range the belt of wet prairie, marsh, and swamp that extends southward from the headwaters of the St. Johns River via the Kissimmee drainage to Lake Okeechobee contains very few islands of oak-pine and constitutes a considerable barrier between populations to the east and to the west of it.

Study of samples of *M. puer* from more than 100 localities distributed throughout the range shows that there is a general southward increase in size and intensity of coloration. The minute differentiation of the central Florida populations is revealed only by the complex structures of the con-

cealed male genitalia. The variations are mostly in three components of the phallus: a ventrally split tube or "collar," a dorsal flap, and, enclosed at the base by the collar, a pair of slender aciculate processes. Each of these three components varies greatly in form and size; the variations in each show a general north-south trend but are semi-independent of one another and exhibit many local peculiarities. The resultant combinations produce numerous and distinctive penial types.

From Alachua County south to Tampa, Winter Haven, Orlando, and Sanford a single penial type prevails, showing little variation. It has a narrowly conical collar with small contiguous apical lobes, an inconspicuous dorsal flap and aciculae that protrude as long gently curved "whips." At the southern end of the range a very different penial type occurs, differing but little on the east and west coasts. It has a thick, short collar, widely open and obliquely truncate at the end, an inconspicuous dorsal flap, and aciculae in the form of two heavy, short, open-coiled "cork-screws" largely immersed in the collar. So great are the differences between these two extreme penial types that it is questionable whether insemination of a female from one end of the species range by a male from the other would be possible.

It is in the middle third of the state, in a zone outlined by Bradenton, Kissimmee, and Eau Gallie on the north and by Fort Myers, Okeechobee, and Fort Pierce on the south, that the differentiation and hierarchic grouping of micropopulations is so strongly manifested. Throughout this region the penial collar tapers from the base and then flares into a pair of divergent apical lobes differing in size and form. In the populations east of the Kissimmee drainage the dorsal lobe is very large, broad, and apically divaricate, while in those to the west it is relatively small and inconspicuous.

Each local population seems to be quite constant in its penial type, every specimen in a sample seeming almost a replica of every other. On the other hand, the samples of 22 out of 27 local populations in this central region were distinguishable on penial morphology from all others. Only at Okeechobee were two rather different penial types found at the same locality.

Uniform clines in Orthoptera.—Clines are common in Orthoptera, and many were described long before a name for them was invented. Being so well known, mention of a few examples will suffice.

(a) Linear clines: The hind wing of the grasshopper *Trachyrhachis kiowa* has a heavy dark band and yellow base in the southeastern United States (*k. fuscifrons*) but is unbanded and colorless in the Great Plains and west to Idaho (*k. kiowa*). In the zone between these areas banding and color both gradually diminish toward the *kiowa* region, but they vary semi-independently and in local populations are quite variable in their individual expression. Populations intermediate in appearance occur from Texas west to California and south into Mexico [Morse (27); Hubbell & Ortenburger (18); Hebard (13)]. The grasshopper *Mermiria maculipennis* and the camel-cricket *Ceuthophilus pallidus* are Great Plains species in each of which there are parallel north-south clines in size and in several partly correlated struc-

tural characters [Rehn (29); Hubbell (16)]. Linear clines extending in different directions through the same population may be seen in *Ceuthophilus brevipes* and *C. divergens* [Hubbell (16)].

(b) Centripetal clines: Where mountains rise from lowlands the populations of higher and lower elevations are connected by convergent (centripetal) clines. The lines of equal change ("isophenes") form closed concentric contours. Such a pattern sometimes occurs in species of wide range in which the relation to environment may not be evident. In *Ceuthophilus uhleri*, for example, correlated clines in four characters extend from a center in northern Alabama out to the periphery, which runs through Iowa, New York, and northern Florida. Upon this pattern is superimposed a north-south size cline. Another species, *Ceuthophilus seclusus*, in the Ozark Region shows a striking deformation of the male pronotum. In every direction away from the Ozarks this character changes gradually or rapidly to the normal pronotal type. Field work accomplished in 1954 completed the evidence originally presented for this conclusion [Hubbell (16)].

The stepped clines of Nomotettix cristatus.—Rehn & Grant (30) have recently analyzed by "inspection and estimate" the geographic variation shown by this widely distributed eastern North American grouse-locust. Unlike *Melanoplus puer*, it has few available taxonomic characters, all of them matters of degree: tectation of the dorsum and elevation, compression and profile of the dorsal crest. The existence of three major regional types (*cristatus*, *compressus*, and *floridanus*) was assumed as already demonstrated, and a "typical" condition for each adopted as a standard. All adults among the 1,851 specimens from 441 localities which Rehn & Grant examined were then classed as "typical" or "atypical" of one of the types, or as "intermediate" between two of them, giving a five-step scale from "typical" to "atypical" and three degrees of intermediacy.

Plotting the results on a map, "typical" *cristatus* proves to occur over most of southeastern Canada and the northeastern and north central United States, with a narrowing tongue that extends along the Appalachian summits to Georgia. "Typical" *compressus* occupies most of the Interior Lowland south of the Great Lakes and Iowa and surrounds the Appalachian tongue of *cristatus* at lower elevations, extending from western Florida north along the Upper Coastal Plain and Piedmont to New Jersey. "Typical" *floridanus* occupies all of Florida and a narrow coastal strip north to Virginia. Zones of "atypical" and "intermediate" conditions separate *cristatus* from *compressus* and *compressus* from *floridanus*, narrow where slopes are steep, as along the Appalachians, and broader where they are more gentle, as in western Pennsylvania and on the southeastern Coastal Plain.

Allopatric discontinuity in the Furcatus group of Melanoplus.—The large, fully winged but sedentary grasshoppers of this group occur only in northern Florida and the Georgia Coastal Plain. Their supposed rarity results from their very restricted habitat, which is the often narrow belt of dense shrubbery on seepage slopes bordering swamps and streams. Their distributional pattern, to decipher which has required many seasons' intensive

field work, is that of an "insular" group of nonintergrading allopatric populations occupying a mainland environment. It is closely paralleled by similar patterns in other groups of arthropods that occur in the same general region.

In the *Furcatus* group three major populations are present, distinguished principally by the distinctive male cerci. The first, *clypeatus*, inhabits the Georgia Coastal Plain between the Savannah and Altamaha rivers, to whose banks it occurs. Its cercus is subspatulate at the apex, with the upper distal angle prominent and the outer margin gently convex-angulate. The second, *furcatus*, has the widest range, from the south shore of the Altamaha river into northern peninsular Florida. Its cercus is deeply forked in most parts of the range and approaches a spatulate form only near Lake George, the point farthest from the other two populations. The third, *symmetricus*, is restricted to the Florida Panhandle; at its farthest eastern point its range is separated from that of *furcatus* only by the two-mile breadth of a sand ridge. Its cercus is undivided, like that of *clypeatus*, but the spatulate tip is nearly symmetrical or its lower angle is the more prominent.

Proof that the Altamaha is the "fence" between *clypeatus* and *furcatus* is furnished by paired collections made at opposite points on the two sides, sometimes very close to the river. In each case *clypeatus* occurred on the north, *furcatus* on the south side, and with a single possible exception there was no evidence that the respective cercal forms are at all affected by the close proximity. The queried exception is a colony of *clypeatus* at Darien in McIntosh County, on the north side of the Altamaha near its mouth. In this series the distal margin of the cercus is a trifle emarginate instead of convex, suggesting a slight approach to the strongly forked condition in the other species. *Symmetricus* and *furcatus* show no morphological approach where they come nearest each other.

The *furcatus* population itself shows striking geographic differentiation. A nearly or wholly disjunct population occurs along the Gulf coast in Dixie and Levy counties, Florida; it has the most distinctively modified cercus of the *furcatus* subpopulations. In the main *furcatus* range the cercal form shows clinal change from one region to the next, connecting four widely different end types. One occurs farthest inland in Georgia, a second in the Okefinokee swamp, and a third in the coastal area east of the swamp (these two being sharply divided by a sand ridge barrier but believed connected to the north and south by circuitous clines), and a fourth around Lake George on the St. Johns River. The intervening regions are occupied by populations with intermediate cercal forms; each local population shows only a small range of variation in cercal type. The only previously published information on this situation is that contained in a generalized map showing the geographic relations of the various populations, with brief explanation, in Rogers *et al.* (31, p. 385).

Mosaic-discordant intergradation in Aptenopedes sphenarioides.—This subapterous grasshopper inhabits Florida and adjacent Georgia and Alabama. Hebard (14), using the variations in six characters, divided it into nine populations. The northern five of these were assembled into one sub-

species, of which one was considered "typical" and the other four "atypic"; the central peninsular three populations were made another subspecies, one of them "typical" and the other two "atypic"; and the single south Florida population was itself treated as a third subspecies. Hubbell (17) has recently analyzed Hebard's data and shown that each of his populations is characterized by a different combination of the variants of the six characters he used; that some of these characters vary clinally, others do not; that no two of them change at the same rate or in the same places; that many other combinations than those he recognized may and some probably do exist; that every additional character discovered provides means for further and more complex subdivision; and that there is indication of the existence in this species of ecotypic phases related to occurrence in certain types of environment. The total picture is one of utmost complexity. *Aptenopedes sphenarioides* appears to exemplify mosaic-discordant intergradation carried to such an extreme that none of its populations remains unaffected by it. The origin of this situation is undoubtedly to be found in the Pleistocene changes that occurred in this region.

THE INTERPRETATION OF GEOGRAPHIC VARIATION IN INSECTS

If the foregoing examples suggest any generalization, it is that *gamma*-1 taxonomy leads only to the threshold of understanding. It raises questions that must be answered by experiment, collateral evidence, or theory, if "explanation" is to be achieved. Some of the needed experiments are so simple and easy to perform that even the practical taxonomist may find time and means to carry them out. The author is chagrined to think that during his years in Florida he was too engrossed in tracing geographic patterns and finding new species to try mating north and south Florida *Melanoplus puer* to see if mechanical isolation does exist between them, as suggested above, or to make the simple tests needed to find out whether *Melanoplus clypeatus* and *Melanoplus furcatus* are or are not reproductively isolated.

Microgeographic variation and its taxonomic treatment.—Fortunately for the practical taxonomist, few species can be so finely subdivided by morphological characters as can *M. puer*. A better example would be hard to find, however, to illustrate the distinctness of local populations and the necessarily heterogeneous nature of all higher taxonomic groupings. Even the finest subdivision based on morphology is almost sure to include several or many genetically different local populations, and the more inclusive the category the more diverse must be its components. The number of discriminant characters used is also important; categories defined by several characteristics will ordinarily contain populations more similar to each other than will categories defined by a single one.

In terms of genetic constitution the hierarchy of populations within the species must include a very large number of levels, but the number of such levels that can be distinguished on the basis of morphological characters is small and depends upon what and how many characters are available and the methods by which they are studied. In *M. puer* five such levels can be

differentiated on the basis of existing data: the primary species population, its three secondary divisions, the tertiary two into which the central population is divided, the quaternary subdivisions of these two, and the quinary division into individual micropopulations. In *Nomotettix cristatus*, on the other hand, the few and relatively indiscriminative characters permit recognition of only two taxonomic levels, the primary species population and its three secondary populations (subspecies). When Rehn & Grant (30) remark upon the "uniformity" of the subspecies of *N. cristatus* throughout their wide ranges they refer, of course, only to these few evident characters. In view of the enormous hidden complexity of the "uniform" species population of *Drosophila pseudoobscura*, the chromosome differences between neighboring colonies which White (38, 39) has described in the only moderately variable *Trimerotropis sparsa*, and much other similar evidence, it seems probable that "uniform" populations are only a taxonomic fiction. Genetic, cytologic, physiologic, or ecologic analysis would doubtless show that the morphologically uniform subspecies of *N. cristatus*, like other populations of like size, contain ecotypic clines, distinctive local populations, and larger populations at various levels below the subspecies.

How is such a situation as that in *M. puer* to be treated taxonomically? A proposal by Mayr seems sensible: to recognize as many subspecific levels and as many groups at each level as are needed for analysis and discussion, but to give formal latinized names only to those of the first (subspecific) level. This still leaves to be decided the actual choice of subspecies. In 1932 the author distinguished in *puer* three subspecies, a northern one quite uniform in penial type (*p. puer*), a southern one, also quite uniform but different in penial type (*p. peninsularis*), and a central one (*p. seminole*), highly variable but showing an average transition in penial type between those of the other two subspecies [Hubbell (15)]. Decision to treat *seminole* as a subspecies was based upon the general aspect of its penial structures, the regional differences in its populations, suggestive of subspeciation, and also no doubt by the desire to have something new to name. Alternatives considered and discarded were to recognize two terminal subspecies connected by a wide zone of intergradation, or else to distinguish two closely related and variable subspecies east and west of the Kissimmee barrier in addition to the more distinctive terminal ones.

As dealt with above, the problem of distinguishing subspecies is largely semantic; the choice made depends not upon evidence but upon judgment and convenience, which according to Mayr are the sole grounds for grouping populations into subspecies. Is there no more objective approach? In this instance one may be found in the variability of the central populations, which is typical of mosaic-discordant intergradation. This strongly suggests that *puer* and *peninsularis* are descendants of populations which diverged in isolation and were later able to spread, meet, and hybridize. What was named *seminole* is, then, the zone of intergradation, and *seminole* is a subspecies of convenience only.

Clinal variation and clinal gradation.—Most clinal change is now thought

to be ecotypic and prevalingly ecogenotypic, the result of natural selection acting upon genetically polymorphic populations [Dobzhansky (8, Chap. V); Ford (11); Sheppard (32)]. The theory is that the gene-complex of each local population is in dynamic balance resulting from the interplay of mutation rates, gene flow, and natural selection and kept responsive to the latter by mechanisms (chiefly heterosis) for maintaining genetic polymorphism [Ford (11, p. 106); Haldane (12, p. 113)]. The sum of the local adjustments, integrated by gene flow, is expressed in a large population as clinal change in characters.

The rate of clinal change is, then, a function of rate of regional environmental change and degree of impediment to free gene interchange; clines are gentle where environmental change is gradual and populations relatively continuous, steep where change is rapid, and variously sloped where partial barriers to gene flow exist. The expression of gradual clinal change is gradation within a population. Many of the characters that show such clinal change seem unrelated to environment, but according to Ford (11) even these are probably always either adaptive or correlated with adaptive characteristics and responsive to natural selection. The relation of regional differentiation and clinal gradation to continuity of gene interchange is well shown in the cited example of *M. furcatus*.

How best to deal taxonomically with gradual clinal gradation in species of wide range is a subject of disagreement. In vertebrate taxonomy and in some insect orders such as Lepidoptera and Orthoptera such populations are treated as polytypic species, with segmental divisions that are considered subspecies. There is no objective way to decide how many and which segments should be so distinguished; proposed rules based on separability of this or that percentage of specimens are purely arbitrary and will give different results depending upon where one begins in the continuum. Another difficulty is that each subspecies includes marginal populations that are both morphologically and genetically more closely related to adjacent populations assigned to other subspecies than they are to other populations within their own subspecies.

On these and other grounds many now advocate abandoning the attempt to divide populations that show gradual gradation into subspecies, and instead simply describing their variation, using graphic and other means, and when necessary for special purposes designating the local populations by symbols or nontechnical names [Brown & Wilson (2); Burt (3); Hubbell (17); Wilson & Brown (40)]. *Mermiria maculipennis* and the two species of *Ceuthophilus*, *pallidus* and *uhleri*, cited above as examples of species showing clinal gradation, illustrate the two methods of treatment. The first was divided by Rehn (29) into two terminal subspecies connected by two broad zones of atypicality and a central belt of intermediacy. The two *Ceuthophilus* species were not divided into subspecies, but their variations were described and graphed, and certain unnamed intergrading "phases" were distinguished [Hubbell (16, 17)].

Stepped clines and zones of clinal intergradation.—It has been noted that

steepening of clines occurs along zones of rapid environmental change and partial barriers to gene flow, such as commonly accompany abrupt changes in topographic relief. At such places several clines are likely to show simultaneous rapid change, and the coincidence of such "steps" is reflected in a zone of clinal intergradation between the populations on either side. The existence of such a zone of intergradation is a most useful guide to the taxonomist in deciding whether subspecies should be erected and where to draw the lines between them. Thus Rehn & Grant (30), when they oppose cline to subspecies antithetically, are contrasting noncomparable entities. They are applying the term cline to evenly gradated populations, whereas it actually refers to character gradients which may of course be gentle, or steep, or variable. The "isophenes" which can be drawn on the map around their plotted "typical," "atypical," and "intermediate" assignments in *Nomotettix cristatus* are diagrammatically clear illustrations of the occurrence of "stepped" clines that coincide to produce relatively narrow zones of clinal intergradation along zones of rapid environmental change.

Species, subspecies, and superspecies.—According to a widely accepted definition, species are reproductively isolated populations; sympatric populations must then be species or they would not retain their identity. Adjoining allopatric populations, on the other hand, generally intergrade and are considered subspecies of a polytypic species. Mayr (24) maintains that the fact of allopatry in the absence of other evidence warrants such assignment. Island populations separated by barriers are so treated by him on the assumption that interbreeding would occur if they were in contact.

The other possibility, that some allopatric populations are actually species and not subspecies, is given more emphasis in a later publication [Mayr *et al.* (26)]. A monophyletic group of very closely related and largely or entirely allopatric species constitutes a "superspecies." The evidence for their specific status may be a degree of morphological difference comparable to that between species in related groups, or contact or slight overlap without interbreeding.

Although superspecies are probably rare in the interiors of continents, they may be quite numerous in archipelagos, and in coastal plains which have been archipelagos. At least in the grasshopper genus *Melanoplus* the author can point to seven groups endemic to Florida and the southeastern Coastal Plain which are either superspecies or are approaching this status. Two of them (*puer-adelogyrus* and *clypeatus-furcatus-symmetricus*) have been described above. In this same region three superspecies exist in the scarab genera *Mycotrupes* and *Pellotrupes*, and others in various genera of insects, crayfish, and spiders. Mayr (25) has recently suggested a genetic mechanism to account for the apparently rapid differentiation which may occur in isolated marginal populations, even those of considerable size, and which seems to have accompanied the Pleistocene fragmentation of ranges that occurred in the southeastern Atlantic Coastal Plain.

The nonintergradation across practically linear barriers, plus the degree of morphological distinctness, suggest that *clypeatus*, *furcatus*, and *symme-*

tricus have nearly or quite attained reproductive isolation. On the other hand the single colony of *clypeatus* already mentioned, located near the mouth of the Altamaha and showing a faint suggestion of *furcatus* influence, could have resulted from amalgamation of a small population of *furcatus* transferred by stream shift into *clypeatus* territory. *Puer-adelogyrus*, on the other hand, is certainly a full superspecies; in addition to various other differences the penial aciculae, small and delicate in *puer*, are in *adelogyrus* enormously hypertrophied into a great pretzel-like structure, and mechanical isolation between the two species is almost a certainty.

In 1942 Mayr (24) suggested that many of the species groups the author had erected in the genus *Ceuthophilus* are really polytypic species, and cited examples. His judgments were based principally on the allopatric distributions shown on maps made by the author, for one of his "groups" includes six species which belong to three distinct groups but which for convenience had been mapped together. Mayr was probably right in some instances, but in most of the groups cited the species are so distinct in all respects that no one who had studied them could have entertained the idea that any two could possibly be subspecies of a single species. It is difficult to say whether any of the groups may be superspecies. Mayr's suggestion is mentioned here merely as an example of the dangers that surround all attempts to interpret results of work in an unfamiliar field.

Hybridization and mosaic-discordant intergradation.—Two examples described above, *Melanoplus puer* and *Aptenopedes sphenarioides*, show mosaic discordant intergradation between their populations. In the first species this occurs over the middle third of the range, between two relatively uniform end populations; in the second the whole species population is completely involved. Here again one sees an effect of the Pleistocene marine transgressions which brought about isolation of populations, divergence, and reunion; in some instances several such sequences doubtless played a part.

Mayr (24) distinguishes between primary intergradation, occurring between allopatric populations of the same species, and secondary intergradation, occurring between distinct species through breakdown of reproductive isolation. Introgressive hybridization, as described by Anderson (1) and Dobzhansky (8) is defined as occurring between species and results in secondary intergradation. The distinction, however, cannot be absolute, for when two formerly isolated and differentiated populations are rejoined, they may have reached an incipient stage of speciation and show varying degrees of reproductive isolation, and phenomena similar to those of introgression may occur in the zone of contact. In the instances cited above, however, there is no evidence to show how far speciation had proceeded before isolation ended, and in relation to them it will be best not to speak of introgressive hybridization even though introgression of characters obviously occurred.

True introgressive hybridization may, however, be the explanation of the following case. *Ceuthophilus nigricans* and *Ceuthophilus thomasi* are closely related but appear to be distinct species [Hubbell (16)]. The range of

thomasi extends from Michigan and Ohio along the Appalachians to Georgia; that of *nigricans* lies east of the Appalachians from New England to Virginia, and extends west across Pennsylvania into eastern and northern Ohio; isolated records from Kentucky and Indiana require verification. In eastern and northern Ohio *thomasi* and *nigricans* behave as sympatric species, but in a limited area in southeastern Ohio populations occur in which some individuals have only *thomasi* characters and others a mixture of *thomasi* and *nigricans* characters, but none have been found which are typical of *nigricans*. This looks very much as though localized introgressive hybridization had occurred between species that differentiated east and west of the mountains during a glacial period. Evidence by which to test this hypothesis is being sought.

The interpretation of evolutionary and faunal history.—Numerous references have been made above to the bearing of Pleistocene events upon the variational phenomena being considered. Deevey (6) has given a very full account of the biogeography of the Pleistocene of North America, but one will find in it little mention of Florida and the Southeastern Coastal Plain. Yet that region underwent a series of submergences and emergences which, through alternately isolating and reuniting populations, made it an evolutionary laboratory of unsurpassed interest. One may find concentrated here many kinds of evolutionary situations, the result of differences in vagility, ecological requirements, and original geographical location of the groups concerned. Geological, ecological, topographical, and historical data may here be used to explain biological situations; but it also works the other way. In fact, in such studies all available evidence must be fitted into a consistent whole. There was, of course, only one actual regional history, and a hypothesis that is set up to explain what happened in one group must be consistent with the evidence from others. A recent attempt at such a reconstruction of the evolutionary history of the beetle genus *Mycotrupes* in this region [Olson *et al.* (28)] has yet to be put to such a test. Its conclusions were found, however, not merely to depend upon but also to offer evidence in support of the hypothesis that land areas have persisted in Florida throughout much or all of the Pleistocene, instead of that area having been completely submerged on more than one occasion. The postulated islands account for survival of the peculiar endemic plants and animals found in peninsular Florida, have been a cause of differentiation through isolation during periods of submergence, and constituted centers from which differentiated populations spread, sometimes to meet and hybridize, during the times of emergence. The hypothesis of persistent land in Florida was thought to be geologically unorthodox, but when it was submitted to Dr. C. Wythe Cooke, the leading authority on the geology of the Coastal Plain, he surprised the authors by confirming its geological probability and welcoming the biological evidence in its support.

SOME PRACTICAL SUGGESTIONS

Among taxonomists who have been trying to apply the concepts of the

"new systematics" to taxonomy on the morphological level, there is general consensus on certain points that can be summarized in a few suggested "rules." In this connection the essay by Smart (35), "Entomological Systematics examined as a Practical Problem," may well be read. The entomologist should: (a) Recognize that although most entomological taxonomy must now and in the future be done on a morphological basis and under a typological standard, the results are improved by thinking of species in terms of populations. (b) Always use the broadest category that will serve the purpose. Thus in the instance of the field cricket, the broad "species" connoted by the name *Acheta assimilis* will suffice for most applied problems and museum collections, even though this "species" is known to be a composite of four or more "sibling species" which can be distinguished by differences in song, behavior, and habitat and which are at least partly reproductively isolated. (c) Use "technical" latinized names only for populations of species and subspecies status; for all other entities for which names are required, whether individual variants, groups of like variants, or populations of lower rank than the subspecies, use colloquial names or symbols not subject to nomenclatorial rules. (d) Make no attempt to distinguish subspecies in populations showing uniform gradation in one or more directions. The variation can be described, graphed, and mapped, and if names are needed for particular parts of the population or for local populations colloquial names may be given, which may well be geographic. (e) Treat as subspecies of polytypic species those subpopulations of continental populations which intergrade by well-defined steps and those island populations for which there is evidence of intergradation with others or which differ from others only slightly. (f) Treat as superspecies groups those allopatric continental populations which come in contact or slightly overlap without intergradation, and those island populations which differ as much as do most species in their groups. (g) Let convenience and simplicity be the criteria, in deciding between otherwise equally logical treatments of a given taxonomic situation. (h) Be cautious in applying *gamma*-2 theory to the explanation of *gamma*-1 results in the absence of supporting evidence.

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ARTHROPOD RESISTANCE TO CHEMICALS^{1,2}

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This review covers three subjects: (a) diversity in reaction to a toxicant as a basic characteristic of a population, the differences between susceptible and resistant strains as revealed by the dosage-mortality curve, and the use of the slope of the log dosage-probit line as a measure of heterogeneity; (b) certain genetic aspects of heterogeneity; and (c) the biochemical mechanisms known to be concerned in resistance to insecticides.

THE HETEROGENEOUS NATURE OF RESPONSES TO TOXICANTS

Since the first report that houseflies in northern Sweden did not respond as expected to DDT in 1946 (143) several hundred similar instances have been noted involving numerous species and many of the newer insecticides. Poor application, lowered quality, improper timing, and other simple reasons undoubtedly accounted for some of the failures in control, but careful comparative tests have revealed genuine changes in the insect's reaction to the toxicant in many cases. The term resistance, which was first popularized in connection with survival of California red scales under heavy dosages of hydrogen cyanide, rather unfortunately has been used of late in connection with all degrees of less-than-hoped-for control. Closer analysis has shown, however, that more than one condition or process is often involved.

Definition of terms.—All living organisms can carry on their vital functions with little or no impairment in the presence of a chemical up to some level of concentration or amount. This level depends, of course, upon the species, the chemical, the method of exposure, and the criterion of effect, but with these fixed it becomes a measure of the important natural resistance or tolerance (as it will be called hereafter) of the species. It is usually necessary further to specify such matters as stage of the life cycle and age within a stage, sex, and sometimes race or strain. Even then the tolerance can be specified only within a range which may be rather wide, but it provides a basic measure of ability to withstand a toxicant with which changes in this ability can be compared.

Tolerance, as used here, is determined by a great variety of factors, e.g., permeability of the integument which is a function of its anatomical structure and thickness, ease of absorption from the digestive tract, patterns of

¹ The survey of the literature pertaining to this review was completed in June, 1955.

² The following abbreviations are used in this chapter: IM ("insensitive mechanism"); ld-p line (log dosage-probit line); PM ("protective mechanism"); SM ("sensitive mechanism").

behavior which affect the degree of contact with a toxicant, and biochemical reactions into which absorbed toxicants enter. Elucidation of the contribution of all such factors to the total tolerance is an important subject which has been explored only slightly, but in this section the over-all effect only will be considered. Tolerance may differ very greatly among various species, but as a rule does not vary much among representatives of a species living under natural conditions in different regions. Some evidence to the contrary has been offered in a few cases, e.g., the citrus thrips, *Scirtothrips aurantii*, from different districts in South Africa is said to have differed strongly in its tolerance for tartar emetic at the first application (122). Several wild and mutant strains of *Drosophila melanogaster* have been found to differ up to about 24-fold in the amounts of DDT in the larval diet needed to reduce emergence to 50 per cent, though none had previously been exposed to the chemical (137). Brown (14) has called attention to the fact that comparison of natural wild populations with laboratory colonies in which inferior individuals have not been eliminated as in nature may give a false impression that the wild group is resistant. This amounts to saying that the tolerance of a species should be taken as that of the typical population existing in its natural condition rather than of inbred laboratory strains.

A second term is needed to denote the added ability to withstand a toxicant which appears to stem from improved nutrition, extra weight, or any other factor usually associated with what may be called extra vigor. A strain developed by breeding only from those individuals which survive exposure to such diverse stresses as extremes of temperature, lack of moisture, abnormal food, or an injurious chemical will have an altered ability to withstand many kinds of stress including exposure to chemicals. This change from the normal tolerance may be called "vigor tolerance" and may either augment or decrease the normal tolerance. An important example to be discussed later is the series of biochemical and genetic changes brought about by selection with one insecticide which give a minor added tolerance for an unrelated insecticide and often notably facilitate the process of selection with the second chemical.

Finally, "resistance" will be used to denote the added ability to withstand an insecticide acquired by breeding from those individuals which survive exposures to that particular toxicant insufficient to wipe out the whole colony. It is not often strictly specific for the compound used in the selection but usually extends to some degree to allied compounds and is dependent upon some behavior in the organism common to the compounds concerned. While this is usually one or more biochemical reactions, such properties of the insect as change in the permeability of the integument to an insecticide may affect resistance as the term has been defined. Instances arise in which the total change in ability of an insect strain to withstand exposure to an insecticide is the sum of vigor tolerance and true resistance. When the necessary data are available a distinction should be made, but in highly resistant strains the vigor effect is relatively very small, and the whole

change from normal may be called resistance without introducing appreciable error. As will be discussed later, one of the debated questions of the present is whether all possible degrees of resistance must be based upon physical or biochemical behaviors already present to some degree in the species before exposure to the toxicant, or whether new mechanisms may arise *de novo* during the course of exposure.

Characteristics of resistance.—The classic examples of resistance to lime-sulfur, lead arsenate, hydrogen cyanide, phenothiazine, and tartar emetic have been fully discussed in earlier reviews (4, 5, 15) which give references to the original reports. These were all noticed first as failures of routine control measures, and the levels of resistance concerned were measured as number of applications or concentration of insecticide required for a somewhat indefinite satisfactory control or as the number of insects left in some predetermined area or as percentage of unmarketable fruit. Occasional data were gotten on percentage of dead and living insects following certain treatments in normal and resistant areas. These field observations were followed in most cases by more precise determinations of resistance involving exposure to known amounts of insecticide under controlled laboratory conditions. The same pattern of field observation and laboratory confirmation, with additional laboratory breeding under exposure to the toxicant in some instances, has been the rule with the recently introduced insecticides. The excellent reviews by Wiesmann (144) and by Metcalf (85) list and discuss the instances in which resistance has been noted with modern insecticides.

It is obvious that resistance is recognizable with certainty when the effect is marked, especially if this can be expressed numerically. Field observations that once successful practices no longer are effective are ample evidence to the practical entomologist, especially if the condition persists through several seasons. It has become standard practice to make laboratory comparisons of insects from the suspected resistant population with others from an untreated area or from a laboratory colony, with mathematical evaluation of the degree of resistance. The cases listed by Metcalf (85) are given in terms of the increase in LD 50, i.e., the amount required to cause 50 per cent mortality in the groups tested.

A conspicuous feature of all compilations on resistance to insecticides is the diversity in degree of resistance and in severity and duration of exposure required to develop it. Thus the common housefly has become practically immune to DDT in several parts of the world, but in India and Ceylon the closely related species, *Musca vicina* and *M. nebulosa*, were about as well controlled after four years' extensive use of DDT as they were at first (57, 100). Similar differences have been found between culicid and anopheline mosquitoes, and only recently has a low degree of resistance been found in the latter group. Considering insecticides rather than insects, the high levels of resistance associated with use of the chlorinated hydrocarbons contrast sharply with the two- to perhaps tenfold effect so far observed with the organic phosphates. There is then a twofold variation, first in the tendency of

insect species to become resistant and secondly, among insecticides with respect to their ability to select out resistant insects, the housefly and the family of chlorinated hydrocarbons, respectively, being the worst offenders.

Troublesome as the presently known cases of resistance undoubtedly are, from the long range viewpoint a more important question is, can a reliable prediction be made from relatively simple experiments as to the resistance which a given species will develop toward a given toxicant when exposed over a number of generations? If potential resistance toward a newly discovered insecticide can be estimated for a number of species against which it appears useful, a considerable saving of money and effort can be made by doing this prior to undertaking the expensive procedure for determining animal and human safety.

Mathematical terms used.—The physical variations among members of a species, which often can be noted at a glance, are matched by equally diverse variations in their unseen physiological and biochemical processes, including reaction to a poison. Thus even in a group selected for uniformity in size, age, rearing, ancestry, and other similar bases of classification, some will be affected by a relatively small amount of a toxicant, others by a somewhat larger, and still others only by a much larger amount. A plot of dosage versus result, e.g., percentage killed or affected in some measurable manner, results in a line which may vary from nearly straight to a very unsymmetrical sigmoid shape. From such a line representing, for example, the response to DDT of flies from some district, it is possible to make an estimate of the LD 50, and the data from some careful investigations have been expressed in this form. Such a procedure is inadequate for two reasons. First, it determines the LD 50 only approximately because interpolation along a curve is uncertain, and secondly, it neglects the information conveyed by the spread of the dosage-mortality relation. After transformation of coordinates to dosage in logarithms and percentage mortality in standard deviations, or probits derived from them, the data in most cases determine a straight line over the central region. From this can be determined both a more accurate LD 50, which is an index of the mean tolerance or the mean resistance of the group tested, and the slope which measures the diversity of response or the heterogeneity of the group toward the toxicant used. If a large sample is used or data from several small ones are combined, the averages approximate those of the species or strain concerned. A special branch of statistics has been developed, the so-called probit analysis, to aid in the calculations and to measure the dependability of the values found (12, 36, 76).

The log dosage-probit line (hereafter called the ld-p line) as just defined is an expression of the reaction of the tested population to the chosen toxicant, e.g., if obtained with wild unexposed insects it gives the tolerance (in terms of the LD 50) of the species, or with individuals from an area treated with an insecticide it gives a measurement of the resistance that has been developed. Determination from time to time will show how the average resistance is varying and how it is affected by any chosen procedure, such as

continued use of the insecticide, substitution of another or use of none at all.

The LD 50, however, is at best a measure of what has already happened and gives little indication of what may be expected. For this purpose the slope is much more informative. A highly homogeneous population, whose ld-p line is accordingly very steep, will offer difficulties in any selection procedure because the range of dosage from very low to very high mortality is narrow. If with care it proves possible to select those of maximum resistance, their fertility often is so low that propagation is impossible (26, 84). In practical field control either most of the population will survive if the required dosage is underestimated or the very few that survive an effective treatment will contribute but little to the next generation because of low fertility and natural ingress from untreated areas. On the other hand, the most casual use of a toxicant against a population having a wide range of susceptibility, i.e., a small slope of the ld-p line, will select out a range of the more resistant individuals. Martin (83) was referring to this situation when he urged that research on the resistance problem should include "selection of insecticides to which a given insect population shows a low degree of variability." The only data upon which such a choice can be made are those resulting from exposure of insects to prospective toxicants, preferably expressed in the ld-p line.

Limitations on use of dosage-mortality data.—At first sight, it might seem that calculation of ld-p lines from the voluminous data on testing of insecticides against various species would yield the information for interpretation of known cases of resistance and prediction of potential ones. Unfortunately, a large part of the published data is useless for these purposes because no definite ld-p line can be drawn. The sexes usually differ considerably in sensitivity to toxicants and use of both gives a flatter ld-p line and an apparent heterogeneity greater than the true value for either sex alone. And if the sex ratio varies from test to test the slope is altered for that reason alone. Also different methods of exposure often indicate quite widely varying degrees of response. Dosage is measured usually in such terms as concentration of a spray, weight of a dust, concentration of a gas, weight of toxicant applied to the integument or injected within the body. Theoretically, the mortalities resulting from any method of exposure will give a measure of the heterogeneity of the group if a constant fraction of the measured dose is accessible to the insects. That is, the slope of the ld-p line should be independent of the units in which dosage is measured, whereas mortality data, such as LD 50's, depend of course upon how dosage is expressed.

It has been noted often that increase in an already large dose of toxicant, used as a residual film or a dust or sometimes as a spray, does not result in appreciable increase in effect because much of the toxicant is not accessible to the insects. The fantastically high LD 50's and resultant low slopes sometimes quoted for highly resistant strains have been criticized as unrealistic for this reason (18, 151). The same objection is applicable to a lesser degree to suspensions and emulsions since pickup of the toxicant often is not

TABLE I
LD 50's AND SLOPES OF LD-P LINE FOR SEVERAL SUSCEPTIBLE AND RESISTANT STRAINS

Species	Strain	Toxicant	LD 50 (or exposure for 50% kills)	Slope	Method of exposure	Ref.
<i>Musca domestica</i>	Canberra	DDT	$\sigma^0 0.052 \mu\text{g./fly}$	6.5	acetone solution, topical	79
	Illinois		$\phi 0.118$	6.5		
			$\sigma^0 1.15$	1.2		
	Roma		$\phi 2.6$	1.2		
		$\sigma^0 + \phi 53.4 \text{ mg./ft.}^2$		3.3	distillate solution sprayed on wall board	53
	Torre Berkeley		$\sigma^0 + \phi 190$	1.65	acetone solution, topical	105
			$\phi 0.054 \mu\text{g./fly}$	3.1		
	Laton		$\phi 0.50$	1.3		
	Super Laton		$\phi 2.5$	1.3		
	Bellflower		$\phi 7.4$	1.2		
	Super Bellflower		$\phi 40 \pm$	1.1		
	Berkeley		$\phi 0.025\%$	2.35	kerosene spray	
<i>Musca vicina</i>	Laton		$\phi 0.19$	1.0		
	Super Laton		$\phi 0.65$	1.5		
	Laboratory (no exposure)	lindane	$\sigma^0 + \phi 0.42 \text{ hr.}$	2.65	fumigation	150
	Laboratory LDD (continuously exposed)		3.8	1.55		
	Ballard (barn sprayed often)		$15 \pm$	1.1		
	Laboratory (no exposure)	dieldrin	$\sigma^0 + \phi 0.67 \text{ hr.}$	1.35		
	Laboratory LDD (continuously exposed)		25 \pm	1.0		
	English (no exposure)	γ -BHC	$\sigma^0 + \phi 0.028 \mu\text{g./fly}$	3.0	oil solution, topical	20
	Yaba (no exposure)		0.014	3.8		
	Ilaro (3 season's spraying)		0.16	2.0		
	Slaughterhouse (no exposure)	tech BCH	4 min.	3.2	plywood sprayed with acetone solution	39

Species	Strain	Toxicant	LD 50 (or exposure) for 50% kill)	Slope	Method of exposure	Ref.
<i>Aedes taeniorhynchus</i>	Quaranfil (2 season's spraying)		80 min.	2.0		
	region not treated, Titus	DDT	4° instar larvae		suspension from	33
	treated 5 years, Cocoa		0.007 p.p.m.	3.8	acetone solution	
	treated 5 years, S. Causeway		0.012	2.6		
	region not treated, Volusia		0.15 ± ♀ 0.31%	1.4 2.9	cyclohexenone so- lution spray	
<i>Calandra granaria</i>	region not treated, Titus		0.37	2.2		
	treated 5 years, Sarasota		2.0	1.9		
	treated 5 years, S. Causeway		10 ±	1.5		
	from 3 English warehouses	Hg vapor	11.0 mg. hr./m. ³ 47.7 55.0	3.71 3.33 2.65	fumigation	11
<i>Blattella germanica</i>	laboratory strain (exposed for 10 generations)		18500	0.50		
	laboratory strain*	DDT	♂ 0.146 gm./l. 0.337	♂ 2.96	dipped in suspen- sion	24, 45, 46
	7° selected generation		0.034	1.72		
	laboratory strain*		0.093	4.42		
	9° selected generation		6.20	2.37		
	laboratory strain*		0.018	3.96		
	11° selected generation		0.260	2.05		
	laboratory strain*		0.013	1.12		
	12° selected generation		0.292	0.69		
	laboratory strain (nonresistant) resistant (from tested area)	chlordane	♂ 0.0035 0.35	♂ 5.3 1.75	dipped in emulsion	48
<i>Chromaphis juglandicola</i>	good field control		♂ + ♀ 20 mg. 140	3.0	5% dust	88
<i>Boophilus microplus</i>	poor field control	parathion		2.3		
	nonresistant	γ-BHC	♀ 40 p.p.m.	3.3	dipped in suspen- sion	59
	Rockhampton		♀ 7400	2.0		

* In each test the laboratory strain and appropriate generation of selected strain were run together.

proportional to its concentration (133). Fumigants, solutions in oil and other relatively nonvolatile solvents (18, 61), light topical applications even in volatile solvents, and finely divided toxicants taken *per os*, are more or less free from this difficulty. However, with these selected methods of exposure, intercomparisons seldom can be made for either toxicity level or slope of the ld-p line. The data for *Tribolium castaneum* exposed to pyrethrum extract on filter paper or in a spray (58) and for female houseflies treated topically with DDT solution or sprayed (105) show that in each case the spray gave a flatter ld-p line indicating greater heterogeneity than with the other method of exposure. With any chosen procedure, the time at which effects are measured must be kept constant, for usually the LD 50 decreases and the slope increases as longer intervals are used. With so many restrictions on the use of mortality data it is not surprising that they have been used but seldom to measure the heterogeneity of populations. The data to be cited later have been selected from work that avoids or minimizes the various objections in so far as can be determined.

For purposes of numerical comparisons slope is defined as the change in probits per unit change in log dosage, i.e., per tenfold change in dosage. This is the same as the coefficient b in the equation for the regression line used by numerous writers and is the reciprocal of the standard deviation. It may be calculated by the principle of maximum likelihood (36), or by the simpler procedure of Litchfield & Wilcoxon (76), but, except when confidence limits also are needed, it may be found most easily from a plot of the data by counting the rise (in probit units) of the ld-p line in going over a tenfold increase in dosage. In all cases of comparison between populations the differences are large enough to be obviously significant, unless noted otherwise and in no case are conclusions based on minute differences.

For reasons of convenience in discussing the ld-p line as a measure of heterogeneity of a population toward a toxicant, a distinction may be made between the central portion extending from about 5 to 95 per cent mortality and the two ends, of which the upper is much more important. The central portion represents the heterogeneity of the bulk of the population which will have most influence on the nature of the progeny, unless only individuals able to survive heavy dosages are left from severe exposure before reproduction can occur. Selection seldom has been carried out so severely that the aberrant individuals beyond say the 95 per cent mortality point alone survived, but the special consequences of such selection will be discussed later in connection with the California red scale and HCN.

Effects of selection upon heterogeneity.—It might be expected that selection from a population by exposure to a chemical so as to eliminate the more susceptible individuals would result in a more homogeneous strain, but the situation depends upon the severity of selection and the possibilities inherent in the population. Table I contains the useable data on tolerance, resistance, and slopes which have been found in the literature for several species exposed to a number of toxicants, though doubtless more data are available. A great

deal has been lost because many writers reported only mortalities at some level such as the LD 50's.

The data are largely limited to DDT and are derived from diverse methods of exposure, in some of which the uniform availability of the toxicant was questionable. It is a striking fact, however, that among seven species and six toxicants there is an unmistakable relation between decrease in susceptibility and increase in heterogeneity toward the toxicant. Susceptible houseflies from Australia (79) and California (105) were found to have about the same tolerance for DDT by topical application of acetone solutions, i.e., 0.052 μg . (σ), 0.118 μg . (φ). English flies (20) are similar (0.20 μg . by topical application of oil solutions), since Busvine (18) found that the LD 50 for susceptible houseflies by topical application of oil solutions was four times as high as with acetone solutions. The similar tolerance of native populations makes legitimate such comparisons as those in Table I, though some of the strains came from widely separated sources. No strains with high tolerance have been reported though a serious error has gotten into the literature regarding the flies from Arnäs, Sweden, which Wiesmann (143) found to be very difficult to kill with DDT. Dr. Wiesmann in a letter (June 24, 1955) states that control was good in that vicinity in 1944 and 1945 but became poor during the 1946 season when he secured the flies which were tested in his laboratory.

The same inverse relation between resistance and slope of the ld-p line holds for houseflies and γ -BHC and dieldrin as shown by the data for *Musca domestica* from Florida (150) and *M. vicina* from Nigeria (20) and Egypt (39). In these experiments the exposure period required for 50 per cent mortality was determined so no LD 50 can be calculated. However, the slopes of the log time-probit lines can be compared with only the assumption that uptake of toxicant is proportional to time of exposure. It is of interest that *M. vicina* was one of the species which did not become resistant to DDT in India within four years.

There is abundant evidence that the "nuisance" mosquitoes have become much more difficult, and sometimes impossible, to control with DDT. The numerous publications contain few data from which ld-p lines can be drawn, but those on adult *Aedes taeniorhynchus* from Florida (33) shown in Table I indicate the usual decrease of slope when resistance is marked. The data for larvae point in the same direction but suffer from the possibility of low availability of toxicant from suspensions. A number of reports have indicated difficulty in controlling anopheline species, but no data appear to be available for deciding if this is accompanied by a change in heterogeneity toward the toxicant.

The development of a strain of *Calandra granaria* highly resistant to mercury vapor (11) and very heterogeneous toward that toxicant is of interest because there appears to be no records showing that the grain beetles become more difficult to control after prolonged use of an insecticide.

Laboratory selection with DDT at the level of about 75 per cent mor-

tality and natural selection during practical use of chlordane both produced highly resistant strains of German roaches (24, 45, 46, 48) which were markedly heterogeneous toward the respective toxicants. It should be noted that the testing technique was changed with each generation and hence valid comparisons can be made only between susceptible and selected strains at each stage. Similar laboratory selection with γ -BHC through seven generations (45) caused only a slight change in mortality or slope of the ld-p line for either sex, but this cannot be regarded as an indication of what would result from more prolonged selection.

The data shown for the walnut aphid, *Chromaphis juglandicola*, were obtained by the vacuum bell jar technique and exposure probably was not closely proportional to dosage of parathion (88). However, the increase in LD 50 and lower slope are clear for the groups taken from fields where control was failing. There have been numerous reports of resistance to organic phosphates by other aphids and by mites, but quantitative data have not been found.

Lastly, Table I contains results obtained with γ -BHC on females of a susceptible and a resistant strain of the cattle tick, *Boophilus microplus*, from Queensland (59). A considerably smaller change in mortality of larvae apparently was not accompanied by an alteration of slope of the ld-p line, but the data are very fragmentary. It would be of interest to compare these results with those obtained with the BHC-resistant tick, *B. decoloratus*, in South Africa, but results with the latter species have been given (142) in a form that does not allow the necessary calculations.

Heterogeneity of severely selected strains.—Numerous experimenters have found that after intensive selection with a toxicant has gone on for a number of generations, the resistance tends to reach a plateau. Examples are, houseflies to several chlorinated hydrocarbons (32) and to parathion (81); California red scale to HCN (74). It is of interest to determine what happens to the ld-p line under these conditions. In a few instances data are available to show decrease in heterogeneity after resistance has reached a high level. In independent researches extending over several years, Lindgren and co-workers (73, 74) and Yust and co-workers (155, 157) found that HCN-resistant California red scales, which had a high LD 50 and rather low slope of the ld-p line, upon severe selection in the laboratory developed super resistant strains of higher LD 50's and steeper slopes. Table II (A) contains the pertinent data.

It may be noted that the resistant strains were considerably more heterogeneous toward cyanide than the susceptible ones, but that further selection in both laboratories raised the LD 50's and decreased the heterogeneity. In Lindgren's experiments with young mature female scales, the further selection did not change the LD 95 appreciably, indicating that the limit of resistance had been approximated. With second molt females Yust found a steady increase in LD 95 at least through 55 selections from the original resistant strain so the limit apparently was not reached for that stage. Much

more data for the years 1938 through 1952 are given by Yust (155), but they merely confirm those given in Table II (A). In tests with second molt male scales Yust found the same decrease and later increase in slope of the ld-p line as selection proceeded, but he warned against placing too much faith in the numerical values since the data usually covered only relatively high mortalities (155).

Table II (A) shows that red scales selected with cyanide react toward hydrogen sulfide exactly as if it were cyanide except that much higher concentrations are needed (158). The same decrease in slope and subsequent increase under intensive selection were found for both second molt males and females. It may be predicted that there is the same or a closely similar basis for the toxic action of the two compounds.

Tattersfield *et al.* (137) have found that *Drosophila melanogaster* selected by spraying the adults with a suspension of DDT in a solution of a wetting and dispersing agent go through the same sequence of increasing LD 50 with decreased slope of the ld-p line and eventually reach a much higher LD 50 but steeper line. Table II (A) gives some averages for three cultures of each group. Several other strains showed the same behavior. The severity of selection, e.g., using 0.01 to 0.05 per cent suspensions, controlled the rapidity in which the stages were reached but did not affect the final condition. If the behavior shown by red scale with hydrogen cyanide and by *D. melanogaster* with DDT is normal whenever selection is long continued, then many other examples will be found when the tests are made, though at present no other data have been found, doubtless because experimenters have so seldom recorded the slope of the ld-p line. There seems to be no reason, however, to expect that the final heterogeneity should be identical with that of the starting population as Tattersfield *et al.* suggest.

In the case of houseflies topical application of DDT in volatile solutions or exposure to dry deposits cannot bring out the effect because of increasing inaccessibility of the toxicant as the dosage is increased. But topical application of oil solutions, exposure to oily deposits, or possibly injection would avoid this difficulty. The possibility of attaining a more homogeneous population by intensive selection with a toxicant will obviously depend upon the characteristics present in the group under selection. The more factors that contribute to resistance the more difficult it will be to pass beyond the condition of wide heterogeneity when all or most of these are exerting their effect to varying degrees. Dosage-mortality data on highly selected strains should be published rather than mere LD 50 or LD 95 values as so many workers have done.

Selection not resulting in resistance.—An increase in slope of the ld-p line also may occur when a susceptible population is selected which does not contain individuals possessing a mechanism for resisting the toxicant. In this event the only result is elimination of the more susceptible individuals so the ld-p line becomes steeper and shifts but slightly toward higher dosages. If vigor factors can become accentuated in the more tolerant individuals, the

TABLE II
CHANGES IN LD 50 AND SLOPE (A) AT HIGH RESISTANCE LEVELS AND (B) AT
LOW RESISTANCE LEVELS

Insect	Strain	Toxicant	LD 50	Slope	Method of exposure	Ref.
<i>Aonidiella aurantii</i>	(A)					
	resistant (from field, bred in laboratory since 1936)	HCN	♀ mature mg./l.	1.80	40 min. exposure in 100 ft. ³ vault	74
	6th fumigated generation		0.18	0.32		
	11th fumigated generation		0.32	2.70		
	16th fumigated generation		0.49	3.70		
	resistant from orange grove		0.49	3.70		
	7th fumigated generation		0.19	2.0		
	12th fumigated generation		0.35	2.7		
	18th fumigated generation		0.43	3.0		
	nonresistant laboratory strain		0.48	3.6		
	resistant laboratory strain		2° molt	5.6		157
	super resistant, selected for 43 generations		♀ 0.07 mg./l.	3.0		
			0.17	6.9		
<i>Drosophila melanogaster</i>	HCN-nonresistant (laboratory strain)	H ₂ S	2° molt	3.25	40 min. exposure in 100 ft. ³ vault	158
	HCN-resistant (laboratory strain)		♀ 8.35* mg./l.	2.50		
	HCN-super resistant (50 to 55 generations selected with HCN)		10.8			
	HCN-nonresistant (lab strain)		28.5	3.45		
	HCN-resistant (lab strain)		σ 4.17	2.65		
	HCN-super resistant (50 to 55 generations selected with HCN)		11.6	2.50		
	wild colony, reared without exposure	DDT	20.1	4.25		
			0.088 mg./l.	5.61	sprayed with 0.015% suspension	137

Insect	Strain	Toxicant	LD 50	Slope	Method of exposure	Ref.
	selected by four sprayings with 0.01% DDT plus 12 to 14 sprayings with 0.015% selected further with 0.02%, then by steps to 0.05% and 6 generations at 0.05%		0.176	2.70		
			0.304	4.89		
	(B)					
	wild colony, not selected	γ -BHC	$\sigma^7 + \varphi 1.6 \mu\text{g./fly}$	3.1	acetone solution evaporated on filter paper	99
	13 ^o selected generation		6.0	ca. 10		
	24 ^o selected generation		6.9	ca. 10		
<i>Blattella germanica</i>	laboratory strain, not selected	γ -BHC	(1951) $\sigma^7 14 \mu\text{g./roach}$	7.5	dipped into suspension	45
			$\varphi 26$	9.6		
	7 ^o selected generation		$\sigma^7 16.3$	7.2		
	laboratory strain, not selected		$\varphi 30$	9.3		
			(1953)			
			$\sigma^7 10$	4.7		
			$\varphi 25$	3.4		
	12 ^o selected generation		$\sigma^7 26$	4.7		
			$\varphi 36$	11.5		
<i>Oncopeltus fasciatus</i>	laboratory strain, not selected	DDT	$\sigma^7 + \varphi 10 \text{ mg./l.}$	1.8	dipped into suspension	47
	17 ^o selected generation		37	2.1		
	laboratory strain, not selected	Toxaphene	$\sigma^7 + \varphi 190$	1.6		
	17 ^o selected generation		345	2.5		

* Recalculated from % by volume.

TABLE III
LD 50's AND SLOPES OF LD-P LINE IN CASES OF CROSS RESISTANCE

Species	Strain	Toxicant	LD 50 (or exposure (for 50% kill)	Slope	Method of exposure	Ref.
<i>Musca domestica</i>	DDT-susceptible	rotenone	$\sigma^3 + \varphi 4.0\%$	1.00	sprayed with hex-	149
	DDT-resistant		5.18	1.55	anone solution	
	DDT-susceptible	Thanite	9.0	3.45		
	DDT-resistant		15.0	4.00		
	susceptible	lindane	$\sigma^3 + \varphi 0.41$ hr.	2.65	air saturated with	150
	DDT-resistant		0.96	4.45	vapor at 80°F.	
	DDT, lindane, dieldrin resistant		2.9	1.55		
	lindane-resistant		14 \pm	1.1		
	susceptible	chlordane	0.57	1.87		
	DDT-resistant		1.1	2.70		
<i>Aedes taeniorhynchus</i>	DDT, l, d, resistant		9 \pm	1.4		
	lindane-resistant		9 \pm	1.4		
	susceptible	dieldrin	0.67	1.35		
	DDT-resistant		1.83	5.04		
	DDT, l, d, resistant		35 \pm	0.8		
	lindane-resistant		35 \pm	0.8		
	susceptible	aldrin	<0.25	2.9 \pm		
	DDT-resistant		0.38	6.8 \pm		
	DDT, l, d, resistant		2.44	2.25		
	lindane-resistant		1.6	1.73		
<i>Blattella germanica</i>	three untreated areas	chlordane	0.16, 0.20,	2.1, 1.6,		33
			0.28	2.7		
	DDT treated area		$\sigma^3 0.85\%$	4.4		
	three untreated areas		0.67, 1.18, 2.2	1.9,		
			1.48	1.2		
	DDT treated area		$\varphi 1.27$	5.0		
	dieldrin-susceptible	TEPP	$\sigma^3 0.05$ ml./l.	4.55	dipped in water	48
					solution	
	dieldrin-resistant		0.112	7.60		
	dieldrin-susceptible		$\varphi 0.153$	3.10		
	dieldrin-resistant		0.265	4.50		

whole line will shift. But in no event can vigor tolerance of itself lead to the marked flattening and general movement toward higher dosages characteristic of true resistance, though no arbitrary dividing line between the two effects can be drawn. Of course, increase in both vigor tolerance and resistance may occur during the first stages of selection, their relative effects depending upon the severity of selection and the character of the population.

Four cases of attempted selection for resistance may illustrate the points just mentioned, cf. Table II (B). Exposure of *D. melanogaster* adults to γ -BHC with 60 to 80 per cent mortality each time raised both the LD 50 and slope (99). Topical application of acetone solutions to the two strains at the twenty-fourth generation gave an increase of LD 50 from 0.0057 $\mu\text{g./fly}$ to 0.0182, but the slope remained practically constant. Injection into the same generation also showed a slight increase in LD 50 but no certain change in slope. Apparently this insect developed no clearcut resistance to γ -BHC by the selection program used but did show a marked vigor tolerance to residual deposits. Selection of *B. germanica* adults with γ -BHC through 12 generations about doubled the LD 50 for males and increased the heterogeneity, but changed the LD 50 only slightly and decreased the heterogeneity with females (45). These results suffer from the previously stressed difficulties with suspensions of toxicants, but they indicate that the German roach developed little if any resistance to γ -BHC. Lastly, selection of the milkweed bug with DDT or toxaphene through 17 generations resulted in an increase in both LD 50 and slope for each toxicant (47). These results also are typical of vigor tolerance. A point of particular interest as yet unexplained, is the fact that this bug metabolizes injected DDT to an unidentified metabolite (35), and it might be assumed that this mechanism could be intensified readily by selection. Data are as yet lacking by which these results may be reconciled.

Cross tolerance and resistance.—Probably the most important cases illustrating the difference between vigor tolerance and resistance have occurred with resistant strains exposed to other toxicants. The term "cross resistance" has been used for all instances when selection for one insecticide led to lowered susceptibility for others. In the light of the foregoing discussion it is clear, however, that a distinction should be made between the effect of vigor tolerance and true resistance toward the toxicants for which selection has not been made. It has been recognized for some time that classification of the modern organic insecticides on the basis of their chemical constitution coincides closely with a grouping according to the intensity of the "cross resistance" (17, 85, 110). Thus six groups may be recognized: DDT and its relatives such as methoxychlor and DFDT; the nitro ethane analogues of DDT, Prolan, and Bulan; the polychlorinated aromatics such as lindane, chlordane, aldrin, toxaphene; parathion and other organic phosphates; pyrethrin and allethrin; the lethanes and other thiocyanates.

Table III contains data for three insect species selected for resistance to one toxicant (in one case more than one) and then tested against others of the same and different groups. It may be noted that when the two toxi-

cants are from the same group, e.g., lindane-dieldrin, chlordane-lindane, dieldrin-lindane, aldrin-lindane, the result is increased LD 50 and lower slope of the selected strain compared with an unselected one. This is to be expected when the second toxicant is chemically similar to the first so that resistance to one confers resistance to the other. On the other hand, when they come from different groups as rotenone (149), Thanite, lindane (150), chlordane (33), etc.—DDT or TEPP—dieldrin (48), the result is typical of vigor tolerance, i.e., increase LD 50 and higher slope. Additional results of this nature have been reported for dieldrin following selection for DDT on houseflies (81).

Synergists.—The purpose of adding a nontoxic synergist to an insecticide is to increase the mortality with a given amount of toxicant, or to reduce the amount needed for a given kill. In a few carefully studied cases of houseflies exposed to DDT it is apparent that at the same time the slope of the ld-p line is successively increased, i.e., the treated organisms act as if they were less heterogeneous in the presence of the synergist. Table IV gives data showing a sharp increase in slope when piperonyl cyclonene was added to a kero-

TABLE IV
EFFECT OF A SYNERGIST UPON LD 50 AND SLOPE OF LD-P LINE

Species	Strain	Synergist	Toxicant	LD 50	Slope	Method of exposure	Ref.
<i>Musca domestica</i>	Super Laton	—	DDT	90.65%	1.55	kerosene spray	105
		0.5% p.c.*		0.20	2.30		
		1.0		0.11	4.7		
	Super Laton	—		2.50 μ g./fly	1.3	acetone solution, topical	
		1 μ g./fly		0.74	1.4		
		10 μ g./fly		0.20	3.0		
	Bellflower	—		7.4	1.3		
		1 μ g./fly		4.6	1.3		
		25 μ g./fly		1.2	1.9		
	Roberds	—		14 μ g./fly	2.47		103
		0.32%					
		DMC		1.3	2.36		
		3.2%		0.45	5.7		
		6.5		0.40	8.1		
<i>Calandra granaria</i>	laboratory strain	—	pyrethrins	$\sigma^7 + 911.3$ p.p.m.	2.30	kieselguhr base dust in wheat	41
		3.75 p.p.m.					
		p.b.†		1.2	3.15		
		7.5		0.8	1.55		
		15		0.55	2.75		

* Piperonyl cyclonene.

† Piperonyl butoxide.

sene spray containing DDT and a smaller effect when acetone solutions were applied topically (105). Addition of DMC [2,2-bis(p-chlorophenyl)-methyl carbinol] in topical applications to the highly resistant Roberds strain increased the slope markedly (103). A smaller but noticeable increase was caused by addition of piperonyl butoxide to γ -BHC upon *Tribolium castaneum* adults (41).

In contrast to this behavior with DDT-resistant flies, in the case of susceptible flies (Berkeley strain) addition of piperonyl cyclonene in small amounts to topical applications did not influence the toxicity of DDT and in larger amounts depressed it and flattened the ld-p line. In sprays it lowered the LD 50's somewhat without effect on the slope (105). Effects similar to these have been found from adding terpin diacetate to pyrethrin spray used in Peet-Grady tests with houseflies (109). Anticipating a later discussion it may be stated that both piperonyl cyclonene (106) and DMC (103) have been shown to strongly suppress the degradation of DDT to DDE in resistant flies. Suppression of a defense mechanism by a synergist would reduce the heterogeneity of a resistant population possessing this factor, thus making it more like a susceptible strain. On the other hand, if the synergist merely makes the toxicant more available, e.g., by decreasing evaporation from small droplets so a flying insect picks up more spray (28) the slope of the ld-p line should be unchanged.

It is tempting to attribute the synergistic action of piperonyl butoxide, piperonyl cyclonene, and other materials when used with pyrethrins to a similar inhibition of some detoxification mechanism, and Wilson (148) has made this suggestion. However, the evidence from the slope of ld-p lines is confusing since both an increase and no effect upon addition of synergists have been reported. In many experiments the availability of toxicant has been a variable for which allowance was uncertain. The whole subject has been reviewed by Blackith (10). When synergists are active with nonresistant organisms they probably always act by making more toxicant available which will not affect the heterogeneity of the population.

In the foregoing discussion the synergists mentioned have all been non-toxic when used alone. In the more general case of mixed toxicants if they are similar in action and one can be expressed in terms of the other, the ld-p lines are parallel. If the toxicants act dissimilarly, in the general case the population is more heterogeneous toward the mixture than toward either alone. This subject has not been explored adequately for resistant strains and is too confused for further consideration here.

Recapitulation.—The foregoing experimental results and predictions concerning the heterogeneity of insects toward toxicants as measured by the slope of the ld-p line under various conditions may be summarized briefly by aid of a diagrammatic presentation (cf. Fig. 1, A and B). Let S in Figure 1 A be the line representing the natural tolerance. If selection results in elimination of weaker individuals without much increase in the proportion having higher tolerance the line will become steeper or move slightly to the

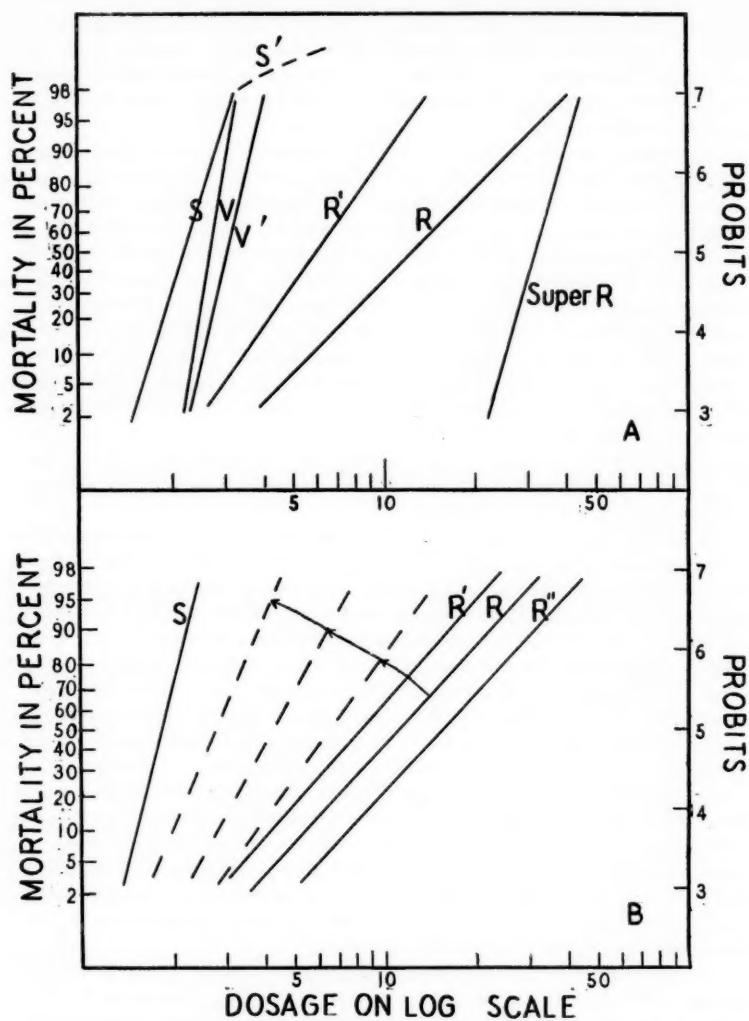


FIG. 1. Log dosage-probit lines corresponding to various situations described in text.

right, e.g., V or V'. Cross resistance to a second toxicant attributable to vigor tolerance illustrates this situation. It also may be the first step in true selection. If a resistant strain can be selected the line moves to the right and the slope decreases (R' , R). But if selection is pressed the line once again becomes steeper as the limit of resistance is approached (Super R). Cross resistance to a chemically related compound, e.g., to methoxychlor in DDT-resistant insects, follows this pattern but usually does not reach the same level. Reversion after exposure is terminated presumably follows these steps in reverse but data are not available. For the significance of S' see page 109.

In Figure 1B, R is the line for a resistant strain and the dotted lines represent the effect of increasing amounts of a synergist which interferes with a defense mechanism. If the synergist acts advantageously in some mechanical manner its presence will result in R' ; if it interferes by decreasing accessibility of the toxicant, i.e., acts as an antagonist, the effect is given by R'' .

Some genetic considerations.—The genetic basis of resistance to insecticides has received attention since the early instances of codling moth larvae-lead arsenate and red scale-hydrogen cyanide, and the multiplication of cases recently has attracted numerous workers. Since this subject is to be reviewed in the second volume of the *Annual Review of Entomology* only a few points directly concerned with changes in the position and slope of the $ld-p$ line will be considered here. Insecticides have not been found to be mutagenic agents, and hence the changes undergone by a population under exposure must result from mutations occurring for other reasons or, much more probably, from the spread of genetic characters already present in a small part of the original population. Selection within any limited group will reveal only the resistance that was potentially available in the group, which may be quite different from that of another group of the same species. For this reason all laboratory selections inevitably reveal less than the total potential resistance of the species.

An important question in any case of resistance is the number of factors which contribute to it. Other circumstances being equal, the fewer factors involved, the more quickly they will become widespread through the population with consequent movement of the $ld-p$ line to a less steep slope corresponding to greater heterogeneity and on to the final steeper slope indicative of a more homogeneous state near the limit of resistance. It should be noted that even a single factor will give rise to a state of considerable heterogeneity, for as Crow (25) has pointed out the variance attributable to one factor goes through a maximum when half the population have it, and the change as shown by the slope of the $ld-p$ line may be several fold. The same considerations apply to a number of factors linked so that they act as a unit. It is commonly considered that the factors conferring resistance are unfavorable in ordinary life, for otherwise the species would be tolerant of the toxicant. But many species are highly tolerant toward chemicals that are harmful to others and hence defense mechanisms need not be deleterious.

Selection programs have shown that there is a lag extending through a

few to many exposed generations during which resistance increases but little. This period is succeeded by one of rapid increase which leads in a few more generations to a limit characteristic of the chemical, the method of selection, and the population (31, 32, 81). A long initial period is to be expected if the resistance factors (*r* genes) are very rare, and rapid increase in resistance is possible only after they spread through a considerable fraction of the population. During the period of rapid increase the slope of the *ld-p* line becomes much less. The slope will increase again, as in the case of red scale selected for cyanide resistance, when the *r* genes are in most of the population. However, this decrease in heterogeneity will not be attainable if the *r* genes carry or are linked with such harmful effects that a balance is reached between their benefits in protecting from the toxic chemical and the weakening which they cause. This appears to be the case with numerous strains of the housefly and DDT since increase in slope of the *ld-p* line with intense selection has not been reported. This condition of balance between good and ill is especially likely to occur when there are multiple genes involved.

Loss of resistance, or reversion as it is sometimes called, is certain to occur in nature if the resistance is acquired at the price of lowered vitality, though under laboratory conditions the decrease may be slow. From the practical standpoint a lasting resistance is possible only when the *r* genes become linked during the period of selection with some vital function. Such a linkage may be very slow to establish but once made it will be equally slow to break. This is what has occurred before exposure to the toxicant in cases of natural tolerance. If during a period of nonexposure the highly resistant individuals are outbred, they can quickly be restored to high proportions so long as the essential linkage is preserved in a few. Some instances of development of resistance during the summer and decline during the season when insecticides are not used seem to illustrate this situation. Because of the much greater possibilities of favorable linkage in large natural populations (27), reversion of resistance arising in the field is less likely than among laboratory populations (111). Hence hopes should not be aroused by such cases of loss of resistance.

The classic procedure of crossing susceptible and resistant strains and measuring segregation of resistance in the F_2 generation has been used with insects in several cases. Harrison (53, 54), using a susceptible laboratory colony of *M. domestica* and a highly resistant colony taken from a treated area in Italy, found that crossing $S\sigma$ with $R\varphi$ or $R\sigma$ with $S\varphi$ resulted in an F_1 generation having resistance to knockdown intermediate between those of the parent strains. In the F_2 generation the segregation of *R* to *S* was almost exactly 1:3, thus indicating that so far as knockdown from DDT is concerned, susceptibility is dominant and a single gene or groups of linked genes controls resistance. A ratio of 1:1 from a backcross of $F_1 \times R$ was additional evidence. On the other hand, with respect to mortality from DDT, the F_1 generation was also intermediate, but the F_2 was very heterogeneous, ranging all the way between the two original parent strains and hence hav-

ing an ld-p line of less slope than either. This indicates multigenic control of resistance to kill by DDT, in agreement with the earlier conclusion of Bruce & Decker (16).

The data of Milani (89) from crosses of two strains (Tripoli and Iran) of moderate resistance or of Tripoli with a highly resistant strain (Roma) show that the progeny were more heterogeneous than either parent strain according to the slopes of the ld-p lines. Crosses of Illinois R with laboratory S gave intermediate resistance to DDT (96), and there was a questionable indication that $R\bar{q} \times S\sigma$ was higher than the reverse. Making the same comparison but with different strains Johnston *et al.* (63) found no evidence of sex linkage, but since in backcrosses resistance was always higher when it came through the female, they concluded that the resistance factor must be cytoplasmic. Lastly, Keiding (64) reported that in the F_1 generation of crosses between susceptible and resistant strains from Denmark there was a 3S:1R segregation and a 1:1 segregation in backcrosses, indicating a single gene controlling resistance to kill by DDT. This is similar to Harrison's finding for knockdown from that chemical but seems to be the only case of this kind so far reported for mortality resistance.

King (65) carried two laboratory strains of *Drosophila*, from the same original wild population, through a selection program by subjecting adults to an aerosol of DDT in tributyrin. The two strains showed the same gain in resistance as judged by time in the aerosol needed to cause 50 per cent mortality. From this it might be assumed that the same resistance factors had been intensified in each. But in the F_2 generation the resistance dropped to half or less and the slope of the ld-p line was decreased considerably, showing a wider heterogeneity just as in Harrison's results with houseflies. However, the total resistance arrived at was only a few fold which may be mostly an effect of vigor tolerance. The greater heterogeneity in the F_2 generation when all genes from both R parental stocks are intermingled may mean that some of the resistance factors in one strain were antagonistic to some in the other, thus reducing the average resistance even though the heterogeneity was increased.

Fairly precise location of the gene(s) controlling DDT resistance in *Drosophila* has been claimed by Tsukamoto & Ogaki (140), who placed it near those for scabrous and vestigial. They also claimed that the same gene controls BHC resistance. This is contrary to all previous findings that these toxicants are in different groups. It is probable that they were considering only vigor tolerance shown toward BHC which had developed DDT resistance.

The classic example of red scale and hydrogen cyanide was shown by the well known work of Dickson (34) and of Yust *et al.* (156) to be controlled by a single gene, or a closely linked set, which is sex-linked. It was found that susceptible strains usually contain a small proportion of quite highly resistant females which give a nearly flat ld-p line at mortalities of about 98 per cent and above (cf. Fig. 1, line S'). Keiding (64) found the same

kind of individuals in one of his so-called susceptible colonies of houseflies, and it is probable that closer examination of the upper part of the Id-p line will reveal many examples. This suggests that by selection of this part of the population it should be relatively easy to change a susceptible into a resistant strain. Lindgren & Dickson (74) subjected five strains of susceptible scales to repeated fumigation which resulted in no appreciable change in two, a semiresistance in one and a conversion of the other two to a typical resistant condition. The interpretation offered is that the aberrant females are heterozygous in that they carry genes for both resistance and nonresistance. In some the *r* genes are linked with an essential biological function and selection soon results in them becoming predominant in the population. But if the *r* genes are not linked in this way, selection cannot result in a resistant population. The changes which Lindgren & Dickson found came about in a relatively few selections, and the recovery of resistance was similar to that which occurs when a resistant strain which has reverted during a period of nonexposure is brought back readily and also to the relatively rapid increase in resistance to a second toxicant after a population has become resistant to another one.

The foregoing brief account of a part of the genetic work bearing on resistance indicates that little is known about even the best studied situations and that much is yet to be done, especially to disentangle the general factors lumped under vigor tolerance and the more specific factors that contribute to true resistance. But basic explanations must be sought in the physiological processes and biochemical reactions in which toxicants take part. Hence the following section is devoted to an analysis of the information on the biochemistry of resistance.

THE BIOCHEMICAL BASIS OF INSECTICIDE RESISTANCE

Mechanisms of resistance are likely to be primarily biochemical. Insecticides kill by interfering with some "sensitive mechanism" (SM) essential to life. The SM may be one enzyme, or a multi-enzyme system, or a lipoprotein membrane, and it may be relatively simple or very complex. The action of the insecticide on the SM may be countered in two ways: (a) by addition of a "protective mechanism" (PM) which somehow prevents the interaction of insecticide with the SM, or (b) by changing or replacing the SM by some "insensitive mechanism" (IM) not affected by the insecticide.

Behavior patterns.—One obvious possible PM is a new behavior pattern which protects the insect from contact with the insecticide. In HCN-R *Aonidiella aurantii*, prolonged closure of the spiracles (30 min., compared to 1 min. in the S strain) was suggested as a resistance mechanism (52); later work failed to confirm these observations (154) and showed that spiracular closure is probably not an important factor in resistance (114). Rapid onset of "protective stupefaction" in the HCN-R strain is a second possible behavioral PM, but the evidence is not conclusive (44, 72, 156). There are many suggestions, usually not supported by sufficient evidence, that resist-

ant insects are more readily repelled or stupefied by insecticides or avoid ingestion or contact in various ways (3, 8, 16, 18, 51, 56, 65, 90, 124, 138).

Impermeability.—A second possible PM is a change in thickness or permeability of the cuticle to decrease penetration by the insecticide into the insect. There is a prevalent belief that heavily sclerotized insects are relatively resistant to contact insecticides, and there is some evidence that natural resistance to pyrethrins and cuticle thickness are correlated (66, 67, 145). It is likely that the high natural DDT-resistance of a grasshopper, *Melanoplus femur-rubrum*, is a result of failure of DDT to penetrate rapidly through the cuticle of the body or the digestive tract, since DDT is very toxic to this insect when injected into the hemolymph (29). The high resistance of the larva of the Khapra beetle, *Trogoderma granarium*, is likewise attributable to the failure of DDT to penetrate through the cuticle (151), probably because the cuticle wax does not dissolve DDT (112). The hypothesis that acquired resistance to DDT in houseflies is attributable to decreased permeability of the cuticle (143) has been disproved, since the rate of penetration of DDT is the same in R and S strains (130, 153), and the R flies are resistant to DDT injected into the hemolymph (9, 18, 81). When very large dosages of DDT are applied to highly resistant flies, penetration rate may become a limiting factor, and a large fraction of the applied DDT may remain unabsorbed (18, 106, 130). The hypothesis that DDT resistance is partly a result of slower penetration and distribution of DDT has been reasserted in more recent work (71) based on the fact that 10 times more DDT moved to the head region of S flies than of R flies, when the same dosage of DDT was applied to the leg region. Since R flies rapidly convert DDT to DDE (which probably is moved much more slowly than DDT within the body of the fly), the data do not conclusively prove that DDT itself penetrates and moves more slowly in R flies.

Detoxication mechanisms.—A third PM is an enzyme system that can quickly destroy the insecticide. Extensive researches on mammals have shown that they have complex detoxication mechanisms that chemically degrade almost all organic compounds (147). The acquired resistance of some bacteria to penicillin is attributable to a penicillin-destroying enzyme (50). The natural resistance of some fungi to certain phenols is a result of a phenol-oxidizing enzyme (115). Until the advent of insecticide resistance, no work of this kind had been done on insects, and among the many suggested explanations for the striking differences in susceptibility of different insect species to insecticides, detoxicating enzymes were usually ignored (15). Nevertheless, it was to be expected that versatile and efficient detoxication mechanisms would exist, having been developed in the course of evolution to protect insects from the poisonous chemicals present in many of the plants and animals on which they feed.

Detoxication of DDT in the housefly.—Most of the work on insecticide detoxication so far has been on DDT-R strains of *Musca domestica*. The analysis of extracts of R flies treated with DDT (5 or 10 μ g. per fly) showed

that 24 hr. after application about 35 per cent of the applied DDT was present as unabsorbed DDT on the external surface of the flies, and 5 per cent as unchanged DDT inside the flies. The internal DDT was widely distributed in the tissues, but somewhat more concentrated in the fat body and gut region (127, 136). Of the remaining 60 per cent of the applied DDT, about 30 per cent could be accounted for as the nontoxic dehydrochlorinated derivative DDE. Most of the DDE was concentrated in the cuticle hypoderm or in the fat body and gut region (75a, 127, 136). The remaining 30 per cent of the applied dose could not be accounted for in most of the earlier work (6, 60, 104, 130). Later work showed that this "lost" DDT could be recovered from inside the flies by very thorough extraction (107), so that at the end of 48 hr. all the applied DDT could be accounted for either as unchanged DDT or as DDE. In earlier studies with radioactive DBrDT, the only metabolite found was DBrDE (151, 152). These facts indicate that the only important DDT-destroying PM in most of the DDT-R strains of *M. domestica* is a mechanism that dehydrochlorinates DDT to DDE. This mechanism is a major factor in DDT resistance, since the degree of resistance is closely correlated with the rate of conversion of DDT to DDE (103). This system is present chiefly in the cuticle-hypoderm (127) and has been isolated and characterized as an enzyme, DDT-dehydrochlorinase (131, 132). The enzyme is not present in detectable amounts in DDT-S strains, or in a dieldrin-R strain. The cross-resistance of the DDT-R flies to TDE and methoxychlor is attributable to this enzyme, which dehydrochlorinates these analogues of DDT also, though more slowly than DDT.

The DDT-dehydrochlorinating PM is blocked by minute amounts of certain structural analogues of DDT such as DMC (108, 126a) and by larger amounts of the pyrethrin synergist piperonyl cyclonene (38, 105). These DDT synergists greatly enhance the toxicity of DDT to R flies, although they never lower the resistance of R flies to the level of S flies (82a, 126a, 134, 135). This indicates that dehydrochlorination is the major, though perhaps not the only, PM in DDT-R flies. This view is supported by the fact that certain analogues of DDT which cannot be dehydrochlorinated (such as dianisyl neopentane and Prolan) are highly toxic to most strains of DDT-R flies (19, 38, 82).

In DDT-S flies, most of the DDT applied externally penetrates quickly and is concentrated in the gut and Malpighian tubules as unchanged DDT (69a). Minute amounts of applied DDT are changed to DDE (75, 106, 151, 152). It is uncertain whether this change is being catalysed by minute amounts of DDT-dehydrochlorinase, since the enzyme cannot be detected in S flies (131). The fact that synergists like DMC, which specifically block DDT-dehydrochlorinase, do not enhance the toxicity of DDT to S flies suggests that they do not contain any specific DDT-dehydrochlorinase. The slow destruction of minute amounts of DDT (and probably of all other insecticides and organic compounds) may be effected in several possible ways: (a) By simple nonenzymatic chemical reactions, such as oxidations and hydrolyses. (b) By the slow action of some of the many specific enzymes

whose primary function is to degrade normal metabolites such as phenylalanine, tryptophan, etc. (c) By the action of specialized detoxicating enzymes, of low activity but very broad specificity, e.g., one such detoxicating enzyme might slowly oxidize any compound containing a benzene ring. The second possibility is most likely, since "specific" enzymes can slowly attack a surprisingly great variety of organic compounds that only vaguely resemble their normal substrate (43).

The larvae of both DDT-S and DDT-R strains of *M. domestica* are much more resistant to DDT than the adults. DDT applied topically penetrates somewhat more slowly into larvae or puparia than into adults and is changed to DDE slowly in the S strains and quickly in the R strains (127, 136). However, large quantities of unchanged internal DDT are tolerated by both S and R larvae and puparia.

So far, no conclusive evidence exists for metabolic degradation of DDT to other compounds than DDE in R flies. The fact that the recovery of DDT (as DDT plus DDE) gradually declined from 76 per cent at 24 hr. after application to 39 per cent at 120 hr. suggested that some other degradation was taking place, either DDT or DDE being broken down to some unidentified compound(s) "X" (106). Later work, using a more thorough extraction technique, showed that the DDT applied to many strains of R flies can be quantitatively recovered (as DDT plus DDE) at 24 hr., and even at 240 hr. nearly 85 per cent recovery is obtained (107). This suggests that DDE is the only metabolite formed from DDT in the first day or two and that DDT and DDE become redistributed in the body of the fly after several days so that it becomes progressively more and more difficult to extract them completely. When DDE is applied to R flies, 75 per cent to 95 per cent is recoverable after 24 hr., but less and less DDE can be recovered on successive days (127, 136); this suggests a possible degradation of DDE to some unidentified compound(s) "X," but redistribution of DDE to loci from which it is not easily extractable remains a possibility. The techniques of extraction and ultramicroanalysis required for the study of insecticide detoxication are very difficult and subject to many variable or constant errors, the latter being often difficult to detect. Even reliable data, when obtained, can be easily misinterpreted. For example, the identification of DDE is often based on the characteristic pink color given by this compound in the well-known Schechter-Haller analysis (121), but an identical pink color is given by three related compounds which might be formed from DDT: the bis (p-chlorophenyl) derivatives of acetic acid, methanol, and formaldehyde (129). Conclusions based exclusively on Schechter-Haller analyses are therefore not definitive. There is ample corroborating evidence based on other techniques (such as paper chromatography) that the compound formed from DDT in DDT-R flies is in fact DDE, but it must be emphasized that the experimental data in some of the published work on insecticide detoxication has limitations that make the conclusions drawn from this work only tentative, not final.

The water-soluble metabolite, DDA, found in the urine of rabbits fed

DDT, does not seem to be formed in houseflies (107, 136); DDA applied to S or R flies can be completely recovered as unchanged DDA after 30 hr. (127). R flies treated with radioactive carbon-labelled DDT do not degrade it to radioactive carbon dioxide (71, 118).

Detoxication mechanisms in other insects and for other insecticides.—Many insects naturally resistant to DDT degrade it to DDE or possibly to other, so far unidentified, metabolites. This has been demonstrated for the milkweed bug, *Oncopeltus fasciatus*, (35), for both S and R strains of the German roach, *Blattella germanica* (7), for the American roach, *Periplaneta americana* (7, 21, 116, 141), and for the grasshopper, *Melanoplus differentialis*, the Mexican bean beetle, *Epilachna varivestis*, and the red-banded leaf roller, *Argyrotaenia velutinana* (128).

Insecticides other than DDT and its analogues are also metabolized by both S and R strains of many insect species. Lindane, the insecticidal gamma isomer of BHC, apparently is broken down to a nontoxic derivative, quickly in a lindane-R strain of *Musca domestica*, and slowly in a lindane-S strain (97); the nontoxic alpha isomer is also broken down quickly by the lindane-R strain and slowly by the lindane-S strain, but the nontoxic delta isomer is broken down much more slowly by both strains (98). This suggests an enzyme system of fairly broad specificity, capable of destroying a variety of chlorinated cycloalkanes. A similar system may be active in breaking down toxaphene and chlordane in DDT-R (Orlando) flies (60).

There is evidence suggesting that a detoxication mechanism is active in the HCN-R strain of *Aonidiella aurantii* (74a). An enzyme, rhodanese, which converts cyanide to thiocyanate, is widely distributed in living organisms and has been isolated from mammalian liver (125, 126).

The fact that strains of insects highly resistant to the organic phosphate insecticides have not yet developed is not attributable to the absence of detoxicating enzymes. A DFP-R strain of *M. domestica* about 10 times more resistant than the S strain has been studied, and the resistance ascribed to a rapid enzymatic destruction of the organic phosphate ester (23). The "aromatic esterase" present in the blood of rats is not inhibited by para-oxon, but hydrolyses it to nontoxic products (1, 91); recent work has shown that a similar enzyme is present in houseflies and other insects (22, 78, 87). The inability of this detoxicating enzyme in insects or mammals to give complete protection from the toxic effects of the organic phosphates is probably a result of the rapid and irreversible action of these poisons on the cholinesterase of the nervous system. The rapidity of detoxication is of great importance. The chlorinated hydrocarbon insecticides react reversibly with the nervous system, and recovery from the toxic effect of the insecticide will occur if the insecticide is withdrawn from the nervous system within a few hours after application; thus, DDT-R flies may be knocked down by large doses of DDT, but will recover within a few hours because the DDT has caused no permanent injury. High resistance to the organic phosphates will require a detoxica-

tion mechanism so efficient that it can prevent any of the insecticide from ever reaching the nervous system.

One curious aspect of the metabolism of the insecticides heptachlor and aldrin is their oxidation to the corresponding very toxic epoxide derivatives (heptachlor-epoxide and dieldrin). Dogs and rats fed heptachlor rapidly convert it to the epoxide (29, 30), and a similar reaction takes place in houseflies (108a), while the American roach apparently converts aldrin to dieldrin (40). This raises the interesting possibility that heptachlor and aldrin are not toxic, but are "activated" to the toxic epoxides in a step of the degradation process. The presence in both mammals and insects of similar mechanisms for epoxidation, and the demonstration that the fat of mammals contains DDE as well as DDT (102), suggests that many of the detoxication mechanisms will prove to be similar in both mammals and insects. However, the fact that the locust and other insects excrete phenol as a glucoside (95, 123), while mammals excrete it as a glucuronide (147), indicates that there will be some differences.

Storage mechanisms.—A fourth possible PM is the storage of insecticide in regions of the insect body where it has no harmful effect (105). It is well known that the highly lipoid-soluble insecticides such as DDT are accumulated in the fat of rats and other mammals (69); mobilization of the fat reserves during starvation releases this stored DDT, which then causes the characteristic symptoms of DDT poisoning (37). An analogous situation may occur in the larvae of DDT-S houseflies reared on media containing DDT; the larvae contain large quantities of unchanged DDT (presumably stored in the fat reserves), which is released during pupation and kills the adult before it can emerge, or afterward (127, 136). Adult R flies can contain relatively large quantities of unchanged DDT (106, 130, 136) or lindane (13). It is likely, however, that storage plays only a minor role in the resistance of adult insects and can account only for a difference of a few fold in the LD 50, such as often occurs between males and females of the same species. It is difficult to design conclusive experiments that will establish the significance of lipoid storage as a resistance mechanism although this has been attempted for the potato beetle larva (68) and the American roach (92, 93, 94).

Higher SM concentration.—A fifth possible PM is a higher concentration of the SM affected by the insecticide, so that more insecticide will be required to cause severe injury. There is no conclusive evidence that a PM of this type has been developed in any R strain so far. Some DDT-R strains of *M. domestica* have about 1.5 times more of the enzyme, cytochrome oxidase, than do DDT-S strains (119, 120), but it is unlikely that cytochrome oxidase is the SM affected by DDT. High concentrations of DDT do cause a partial inhibition of cytochrome oxidase *in vitro* (62, 119, 120), but the nontoxic *o-p'* and *o-o'* isomers of DDT have the same effect (23), as do also DDE and many nontoxic DDT synergists such as DMC (2, 62). It has never been shown that insects severely poisoned by DDT have a lower level of cyto-

chrome oxidase activity than normal insects; and the highly specific toxicity of DDT to nerve cells also argues against cytochrome oxidase as the primary SM to DDT, since cytochrome oxidase plays a vital role in muscle and other cells insensitive to DDT. In the HCN-R strain of *Aonidiella aurantii* there is no evidence that the level of cytochrome is higher, since the content of iron is the same in the HCN-R and HCN-S strains (50a). The enzyme cholinesterase is not significantly higher in DDT-R than in DDT-S strains of *M. domestica* (80, 113), but this is not surprising, since DDT does not inhibit cholinesterase; however, cholinesterase is no higher in a DFP-R strain, although DFP does inhibit the enzyme (23).

Higher level of secondary biochemical mechanisms, "Vigor."—A sixth possible PM is a higher level of some mechanism not primarily affected by the insecticide, but indirectly affected. The primary effect of DDT on nerve cells is not yet comprehended in detail (42, 49, 70), but a well-recognized secondary effect is intense hyperexcitation of the nervous system which causes violent muscular convulsions (117, 139). This muscular activity causes, as a tertiary effect, a striking increase in oxygen consumption (48, 55, 77). It is at this point in the complex sequence of events induced by DDT that the increase in the level of cytochrome oxidase found in DDT-R flies (119, 120) is likely to exert a protective action, since insects with high cytochrome oxidase can presumably meet the heavy oxygen demand with less danger of oxygen starvation and the resulting quaternary injury effects that may be the ultimate cause of death. This nonspecific PM is one of the many that can be included in the widely-used but ill-defined term "vigor," which is the basis of "vigor tolerance" discussed in an earlier section.

"Vigor" may be defined as the average activity of all the secondary biochemical mechanisms that strongly affect or are affected by the primary mechanism injured by a toxic agent. If this average activity is high, the organism can withstand and recover from the primary injury, since this causes less severe secondary damage. Resistance conferred by high vigor will be relatively low and unspecific. "Vigor tolerance" to one toxic agent will not necessarily confer tolerance to all toxic agents and may even confer susceptibility to some agents.

Decreased sensitivity of the SM.—The simplest possible IM would be an SM altered slightly to be less sensitive to the insecticide. There is no evidence that an IM of this type has been developed by any R strain so far. The fact that parathion is toxic to both the housefly and the honeybee, while the isopropyl analogue of parathion is toxic to the fly and nontoxic to the bee (86), suggests that one of the biochemical mechanisms in the bee is slightly different from that in the fly and that this slight difference makes the bee highly resistant to the isopropyl analogue; the difference is probably in the esterase enzyme system, but may be in the activating enzyme system that converts thiophosphate esters to phosphate esters, or in a detoxicating enzyme system. The theoretical possibility of altering an SM to an IM is also

supported by the discovery of cyanide-insensitive cytochrome b_5 in the larva of *Platysamia cecropia* (101); this respiratory pigment must differ in some way from the other heme-proteins that function in the uptake of oxygen, for it is a terminal oxidase not inhibited by carbon monoxide or hydrogen cyanide. However, mutations that alter an SM to an IM may be exceedingly rare, and this theoretically possible resistance mechanism may never develop in actuality. The cholinesterase in a DFP-R strain of *M. domestica* was found to be identical with that of the DFP-S strain (23). The possibility that HCN-R *Aonidiella aurantii* may have a cyanide-insensitive cytochrome system has not been investigated. With other insecticides, the nature of the SM is not surely known, and so it is not possible to check its relative sensitivity in R and S strains.

"By-passing" of an SM by an IM.—If two parallel biochemical pathways, one an SM and one an IM, are doing essentially the same work, resistance will exist or develop if the IM can take on a larger share of this work, thereby "by-passing" the SM. This type of resistance is common in microorganisms, which possess very complex biochemical mechanisms; but it may be relatively rare in insects, which are more specialized. The classic example of alternate pathways is the existence, in most living organisms, of three major enzyme systems for the uptake of oxygen in respiration: the iron-containing cytochromes, the copper-containing oxidases, and the flavo-protein oxidases. The metal-containing enzymes are usually inhibited by hydrogen cyanide, hydrogen sulfide, and other poisons that form complexes with iron and copper; the flavoproteins are not. The pupal stage of *Platysamia cecropia* is highly resistant to HCN; the level of metal-enzymes is low and respiration is largely mediated by the flavoproteins; during metamorphosis, the level of cytochrome rises steeply, and the insect becomes very sensitive to cyanide (146). There is strong evidence that a similar, though partial, shift from cyanide-sensitive to cyanide-insensitive respiratory enzymes is a major factor in the HCN-R strain of *Aonidiella aurantii* (158).

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THE MODE OF ACTION OF INSECTICIDES¹

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DICHLORO-DIPHENYL-TRICHLOROETHANE

The mode of action of DDT is not known, although it has been the subject of numerous experiments and a great deal of speculation. Much of the excellent work of Riemschneider & Otto (1), Rogers *et al.* (2), Stringer, Woodcock & Skerrett (3), Gunther and co-workers (4), and others on the requisites of the DDT-type molecule cannot be reconciled until something specific about the biological action of DDT is known. Quantitative toxicology and the use of poisoning symptoms as criteria of DDT-like activity cannot be depended upon to fully elucidate the structural requirements of the molecule. Species variation in response to a series of analogues is so great that all hypotheses appear to fall short of a satisfactory explanation. One suspects that many of the discrepancies may be attributable to differences such as absorption, detoxication, and transport to responsive sites within the insects. An *in vitro* method for assaying DDT-like activity, independent of these variables, is essential for the clarification of all the work done in an attempt to elucidate the structural requirements of the molecule.

Enzyme inhibition.—Studies of the effect of DDT on various enzyme systems have all failed to account for the toxic action of DDT. Johnston (5) and Morrison & Brown (6) show DDT to have an effect upon cytochrome oxidase *in vitro*, but this same effect may be obtained with nontoxic derivatives of DDT and would, therefore, seem unrelated to the poisoning process. A report by Stegwee (7) that DDT inhibits cholinesterase is apparently the result of an error in technique, for other workers, Tobias, Kollros & Savit (8), and Richards & Cutkomp (9) have clearly demonstrated that inhibition does not occur, even though at the prostrate stage of poisoning an increase of free acetylcholine or its equivalent in nerve tissue may be as much as 300 per cent above normal (8). A complete explanation of this seemingly incongruous situation has not been made. If this represents the true *in vivo* picture, then it would appear that the additional acetylcholine is somehow unavailable to the enzyme acetylcholinesterase. Lewis (132) suggest that the excess acetylcholine may have arisen through *in vitro* synthesis, but this would require an explanation of why *in vitro* synthesis of acetylcholine did not occur in the controls.

The inhibitory effects of DDT on carbonic anhydrase were reported by Torda & Wolf (10) and Keller (11). Anderson & March (12) concluded from their studies that inhibition of carbonic anhydrase by DDT could not ac-

¹ The survey of the literature pertaining to this review was completed in June, 1955.

count for the poisoning effects of DDT in insects. Anderson, March & Metcalf (13) found that DDT in high concentrations will inhibit succinoxidase derived from houseflies, but this system is also inhibited by nontoxic analogues in the same concentration. Therefore, they concluded that this offers no explanation of the poisoning action of DDT.

Physical basis of action.—Lack of evidence for the effect of DDT on known enzyme systems has suggested that DDT may exert its effect through interference with membrane permeability. Welsh & Gordon (14) demonstrated that DDT and other similar toxic compounds affected a nerve axon of *Periplaneta* in such a way that a single stimulus produced a prolonged burst of impulses. They propose that substances, such as DDT, having a high lipide to water solubility ratio are absorbed upon the lipoprotein surface of the nerve where they may interfere with the stabilizing action of divalent cations. In support of this proposal, Gordon & Welsh (15) showed that repetitive discharges, characteristic of DDT applied to the motor axon of a crayfish were similar to effects produced by calcium binding agents such as citrate or oxalate. It had previously been suggested by Luger *et al.* (16) that lipide soluble materials having hydrophobic properties produce tremors by dissolution of the lipide membrane which would alter its permeability and thus cause the disturbances reflected by DDT poisoning. Such explanations seem to be an over simplification of the problem, for they do not take into account the fact that a number of derivatives of DDT have similar lipide solubility yet are nontoxic, as pointed out by Metcalf (77).

Gavaudan & Poussel (17) propose that DDT acts as an indifferent narcotic because its thermodynamic activity falls within the range postulated by Meyer & Hemmi (18). The symptoms of DDT poisoning though are quite the opposite of narcosis. These workers also fail to explain the inert nature of other compounds and derivatives of DDT which have similar thermodynamic properties.

Mullins (19), recognizing some of the shortcomings of the physical explanation of narcotics and the opposite effect of convulsants, has reviewed the hypothesis of Meyer & Hemmi and its extension by Ferguson (20) into his concept of chemical potentials. Mullins proposes, without cytogeographical reference, a model membrane composed of a lattice of cylindrical lipoprotein molecules oriented in such a manner as to provide free interspaces for the passage of ions and small molecules from one side of the membrane to the other (Fig. 1). A foreign molecule entering the interspace of the membrane lattice will have a narcotic effect relative to its thermodynamic activity provided that the foreign molecule merely enters the interspace without causing a distortion in the three surrounding lipoprotein molecules. Under these conditions a depressed effect is accomplished by temporary interference with membrane permeability. If, however, a foreign molecule is provided with attractive forces properly distributed and can orient itself in the interspace in such a way as to bring these forces into play with the surrounding lipoprotein molecules, then distortion of the interspace may occur.

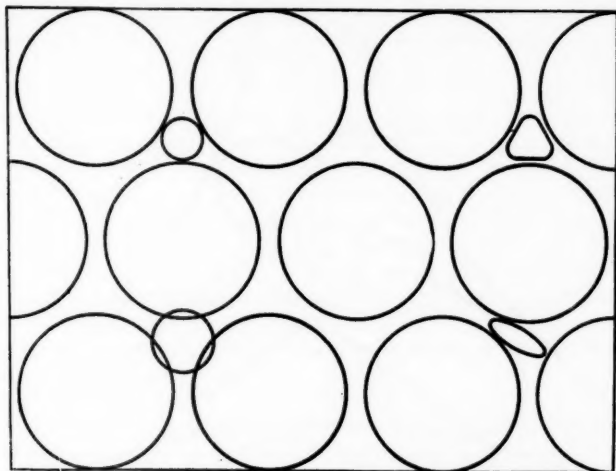


FIG. 1. A model membrane consisting of a lattice of cylindrical lipoprotein molecules oriented so as to allow passage of ions and small molecules. Large circles represent membrane molecules viewed normal to the cell surface. Upper left, lindane in plane orientation and upper right, lindane in an end-on orientation. Lower left, β -isomer is excluded from penetration in a plane orientation, but not (lower right) in an end-on orientation. (Diagrammatic and not to scale.) [From Mullins (19).]

The effect would be that of increasing the size of the surrounding interspaces which would lead to ion leaks and excitation.

Mullins (21) tests the suitability of his hypothesis to explain the mode of action of some of the chlorinated hydrocarbon insecticides. He uses as a model a membrane lattice composed of lipoprotein molecules spaced 2 Å apart and having such diameter that one can inscribe a circle 8.5 Å in diameter in the resulting interspace. A DDT molecule, constructed from Stuart models and imposed in the interspace drawn on a comparable scale, shows that the DDT molecule can enter the interspace only in an end-on orientation as shown in Figure 2. This orientation also brings the attractive forces of the halogens into the most favorable position to be effective. This position, however, is dependent upon the restrictive effect of the ethane chlorine atoms upon rotation of the benzene rings. It is also dependent upon *p*-chlorine substituents which make the axial distance great enough to prevent a plane orientation of one of the rings in the interspace. Thus a non-toxic compound like DDE [1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene] where constraint on ring rotation is relieved, has a greatly increased distance through the axis of the molecule from *p* to *p'* chlorines and cannot orient in the proper manner. Other analogues such as *p*, *p'* iodo or propoxy are too large and penetration is delayed or inhibited.

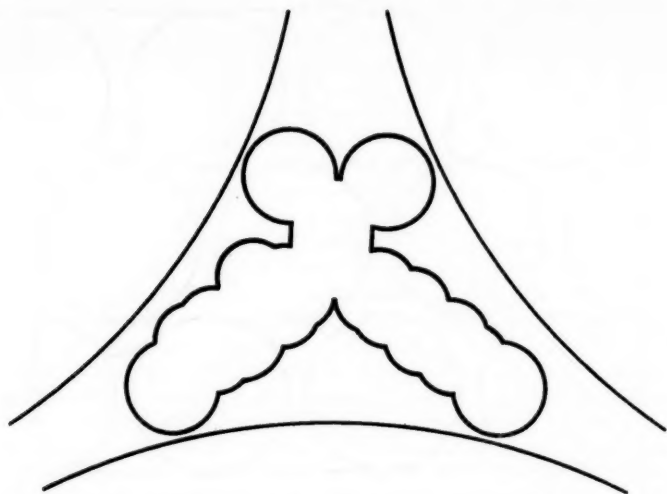


FIG. 2. The fit of DDT into an interspace of the same size as that shown in Figure 3. The benzene ring on the left has been rotated slightly to show how the profile of the molecules broadens. [From Mullins (21).]

Mullins' explanation of a physical basis for the action of DDT requires direct proof which is difficult, if not impossible, to provide by techniques presently available. His hypothesis would, if acceptable, explain many problems pertaining to the cause of species variation which may not be associated with such factors as detoxication and cuticular penetration. For example, one could explain lack of toxic action on a species as being attributable to an unfavorable size in the interspace in a critical membrane. The lack of toxicity of DDT and the high toxicity of methoxychlor to *Epilachna* could be explained on the assumption of an interspace of slightly different size and more favorable to methoxychlor, etc.

Site of action of DDT.—Roeder & Weiant (22, 23) showed by oscillographic methods that DDT applied in low concentrations to the peripheral sense organs of *Periplaneta* had an unstabilizing action on these organs. This instability typically resulted in the generation of trains of impulses instead of the normal pattern of afferent activity. The insensitivity of the isolated central nervous system to DDT led to the conclusion that the principal site of action of DDT is the peripheral sensory system. By a variety of surgical procedures applied before and after the application of DDT to *Periplaneta*, Tobias & Kollros (24) arrived at essentially the same conclusion; namely, that in poisoning with low dosages of DDT, the hypermotor effects result from afferent impulses reaching the ganglia from which they are transmitted through an intact reflex arc to motor fibers.

Le Roux & Morrison (25) applied topical dosages of C^{14} labeled DDT to various areas of the body of the housefly and correlated poisoning with the appearance of about 3 per cent of the $1\frac{1}{2}$ microgram dose in the head. Dosages applied nearer the region of the head, for example, the labella, were more effective than those applied to the legs. The minimum dosage was required when direct application was made to the internal tissue of the head. From these experiments they concluded that the locus of action of DDT was in the head. This is contradictory to the earlier findings of Tobias & Kollros (24) that decapitation of *Periplaneta* before or after DDT poisoning had no effect upon the abnormal activity of poisoning. A similar experiment can be made on houseflies decapitated, treated on the mesonotum with DDT, and placed in an atmosphere nearly saturated with water. Under such conditions the treated flies will show the characteristic hyperactivity, tremors, and terminal convulsive movements quite independent of the fact that they lack heads, according to Lovell (26).

The fact that minimal dosages are required when DDT is applied to the head and its internal tissues may simply mean that there are greater numbers of sensory organs located on the head. Hodgson & Smyth (27) have shown that peripheral sensory organs are responsive to DDT internally transported or topically applied. They have also established that the labellar chemo-receptors of flies are highly sensitive to DDT.

Incidental to their studies on the locus of action of DDT, Le Roux & Morrison (25) found DDT applied internally into the head capsule of DDT-resistant flies was less effective in producing mortality than when applied in the same manner to susceptible flies. From this experiment, they, like previous workers [Babers & Pratt (101)], concluded that nerve tissues of resistant flies were different in sensitivity to DDT from those of susceptible flies and that this difference may not be accounted for by detoxication of DDT. These experiments do not exclude the possibility of detoxication as the cause of the observed difference. It is quite possible that the difference in sensitivity is actually a result of detoxication, because Miyake & Kearns (28) have found the enzyme DDT-dehydrochlorinase [Sternburg, Kearns & Moorefield (29)] present in nerve tissue of resistant flies; whereas, it is not present in measurable quantities in nervous tissues obtained from susceptible flies.

DDT-metabolism in insects.—The first studies on the metabolic fate of DDT in insects were carried out on the large milkweed bug, *Oncopeltus fasciatus* Say by Ferguson & Kearns (30). The milkweed bug is naturally tolerant to topical and injected doses of DDT. It was found that sublethal doses of DDT injected in acetone solution were rapidly metabolized to products which did not respond to the Schechter-Haller (31) test. Metabolites of DDT, namely, DDE and DDA [bis (*p*-chlorophenyl) acetic acid], the principal products of DDT metabolism in mammals [White & Sweeney (121)] were not found in the milkweed bug. Injected doses of DDE appeared to result in some chemical modification of DDE as indicated by the presence of an unidentified compound which showed a different absorption maximum

in the ultraviolet range. Insects injected with DDA were unaffected, and the compound could not be recovered and determined by the Schechter-Haller test. Massive doses of DDT, which produced poisoning symptoms and eventual death, apparently inhibited the mechanisms by which DDT was rendered unresponsive to the Schechter-Haller test, because DDT could then be recovered in quantities approaching theoretical.

Vinson & Kearns (32) found *Periplaneta* could metabolize DDT to DDE and to other products which did not respond to the Schechter-Haller test. They concluded, however, that the ability to metabolize DDT was not the only mechanism in this insect which prevented intoxication. Sternburg & Kearns (33) found that DDT administered as oral dosages to *Epilachna* larvae caused intoxication which persisted as long as measurable quantities of DDT remained in the insect. When the conversion of DDT to DDE and unidentified products was complete, the insect recovered. They concluded that detoxication might account for the tolerance of this insect to DDT. Grasshoppers fed massive oral dosages of DDT excrete large quantities of the dose unchanged. Measurable quantities of DDE may be found in various other tissues of the body, particularly the cuticle-hypoderm. Unchanged DDT appears restricted to the intestinal tract and excreta. Tolerance to DDT in this insect was believed by Sternburg & Kearns (33) to be attributable to the combination of detoxication and the excretory facility of the insect. Winteringham (34) found *Trogoderma* larvae, which are naturally tolerant to DDT, do not effectively modify DDT. Thus, it must be concluded that the causes of natural tolerance are varied and the metabolic pathways of DDT are diverse in different species.

Inhibition of DDT detoxication systems has been restricted to the study of effects of certain compounds applied simultaneously or in sequence with DDT to resistant strains of houseflies. Perry & Hoskins (35) were the first to show that DDT applied in mixture with piperonyl cyclonene [3-isoamyl-5-(3,4-methylenedioxyphenyl)-2-cyclohexene-1-one] to resistant flies resulted in a potentiation of the insecticidal activity of DDT and at the same time reduced the ability of the fly to detoxify DDT. Summerford *et al.* (36) and March, Metcalf & Lewallen (37) found various nontoxic analogues of DDT to be even more effective as synergists for DDT against DDT-resistant flies than piperonyl cyclonene. Of particular interest is the fact that these so-called synergists do not potentiate the action of DDT against susceptible flies. Perry, Mattson & Buckner (38) propose that the synergist DMC [di(*p*-chlorophenyl)methylcarbinol] acts by competitively interfering with the mechanism which the resistant fly utilizes to detoxify DDT. Since their experiments were necessarily carried out *in vivo*, their proposal might be questioned as to whether this is actually the cause or merely the result of synergism.

Moorefield & Kearns (39) tested a number of the analogues of DDT which have been reported to act as synergists on the enzyme DDT-dehydrochlorinase obtained from resistant houseflies. All the synergists tested proved

to have the property of inhibiting the enzyme in concentrations which were practical to test. They also found DMC to be an effective inhibitor of the enzyme and thus confirmed the predictions of Perry, Mattson & Buckner by *in vitro* methods. Moorefield & Kearns conclude that if the only effect of these synergists is to inhibit DDT-dehydrochlorinase then it is logical that they would not potentiate the action of DDT on susceptible flies because the latter have no measurable amount of DDT-dehydrochlorinase.

The metabolic fate of DDT in susceptible and resistant houseflies has been the subject of a number of studies which have been competently reviewed by Metcalf (40) and require no further elaboration here.

Effect of temperature.—The discovery by Lindquist, Madden & Schroeder (41) that houseflies are more susceptible to DDT at low rather than high temperatures provided incentive for other workers to explore this aspect of the mode of action of DDT. At least seven additional species of insects studied all show a clear cut negative temperature coefficient [Guthrie (42); Potter & Gillham (43); Pradhan (44); Hoffman & Lindquist (45); Fan, Chen & Richards (46); Häfliger (47); Vinson & Kearns (32); Munson, Padilla & Weissman (48)]. It was reported by Fan, Cheng & Richards that *Aedes aegypti* (Linn.) larvae demonstrated a negative temperature coefficient to DDT applied as a suspension but had a positive temperature coefficient to injected dosages. This led to the suggestion that the negative temperature coefficient is a result of the greater DDT-concentrating action of the insect cuticle at lower temperatures. Other workers have shown with other insects that a negative temperature coefficient may exist when the cuticle is bypassed by injections [Woodruff (49); Vinson & Kearns (32); Munson (50)].

These findings and numerous other observations suggested the generalization that insects susceptible to DDT may be expected to show a negative temperature coefficient. Any apparent exceptions may be the result of such factors as the criteria used to measure intoxication, range of dosage, range of temperature, methods of application, absorption, etc. The response of DDT-treated insects to temperature seems intimately and fundamentally associated with the mode of action of the compound.

Hurst (51) found blowfly larvae, *Calliphora erythrocephala* (Meig.), are extremely resistant to DDT at 20°C., but if treated with DDT and exposed for 2 hr. at 36°C. and brought back to the low temperature, they quickly show poisoning symptoms. Poisoning symptoms could be reversed by again placing the larvae at the high temperature. Hurst suggested that this phenomenon represented an equilibrium condition in which DDT present in the cuticular lipides could act on the peripheral nervous system only when it is dropped out of solution by chilling. The implications are that the cuticular lipides bar the passage of DDT to its sites of action at the low temperature, but facilitate its passage and act competitively as a storage reservoir with the site of action at the high temperature.

The phenomenon of the reversibility of DDT poisoning symptoms in *Periplaneta*, with changes in temperature, was studied by Vinson & Kearns

(32) in relation to absorption and metabolic fate of topical and injected doses. They showed that DDT was metabolized at both 15° and 35°C. The rate of metabolism was faster at the higher temperature, and the metabolism of DDT diminished with the onset of poisoning symptoms at either temperature. Still a correlation of data on the reversal of symptoms and the metabolism of DDT led to the conclusion that DDT metabolism is incidental, or at most a minor factor as a cause of the negative temperature coefficient of action. Therefore, an explanation must be based largely on the effect of temperature on the intrinsic susceptibility of some physiological system to DDT.

Munson, Padilla & Weissman (48), using their own experiments as a basis and in consideration of the literature on the subject, proposed that insect lipides, as a whole, are more affected by temperature than are the particular lipides present at the site of action of DDT. They assumed that lipides involved with the nervous system may be considered as "elementally constant," whereas changes in neutral fat and free fatty acids in other body fats could occur as an effect of temperature. In support of this proposal they cite the findings of Munson (50) who reports that the iodine numbers fall for total insect fat when *Periplaneta* are held at temperatures above 27°C. Experimentally they have shown that roaches held at 23°C. previous to treatment with DDT do not, by comparison with roaches held at 34°C., reveal a negative temperature coefficient. The conclusion drawn from this evidence is that roaches held at the low temperature have lipid systems of higher unsaturation value and thus greater solubility and holding capacity for DDT than roaches preconditioned at higher temperatures. These workers conclude that an extension of the previously stated hypothesis of Hurst (51) to include internal body lipides is sufficient to explain the negative temperature coefficient of toxic action of DDT.

This hypothesis fully stated would imply that the cuticular lipides act to facilitate the entry of DDT into the body of the insect and at the same time to protect the insect by holding a quantity of the material in storage away from the site of action. The same functions assigned to the internal lipides then, would allow for no evident effects to occur unless these lipid systems became supersaturated and dropped their excess of DDT in such a manner as to become available to the site of action. A drop in temperature could precipitate this condition, and it is tempting to consider this an adequate explanation of the negative temperature coefficient of toxic action.

Other points to be considered in the explanation of the negative temperature coefficient of DDT action are raised by the finding of Sternburg & Kearns (52) that the haemolymph of *Periplaneta* in the prostrate stage of poisoning contains toxins other than DDT. The appearance and disappearance of the toxin in the blood correlates with the reversible effects of temperature on appearance and disappearance of poisoning symptoms. The nature of the toxin is not known.

HEXACHLOROCYCLOHEXANE

Slade (53) suggested that lindane, the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane may be isosteric with the B-vitamin *meso*-inositol and thus block some vital process. Strong supporting evidence for this hypothesis was furnished by Kirkwood & Phillips (54) through their finding that lindane produced partially reversible inhibition of growth in the Gebruder Meyer strain of the yeast, *Saccharomyces cerevisiae*, which requires an exogenous source of *meso*-inositol for growth. Others have since reported similar results obtained on different organisms; Buston *et al.* (55) for *Nematospira gossypii*, Tirunarayanan & Sarma (56) and Fuller *et al.* (57) for *Neurospora crassa*. Sarma (58) found that the larvae of *Corcyra cephalonica* fed on a diet containing lindane had abnormally high cholesterol contents which could be reduced by the addition of inositol to the diet. Roaches [*Periplaneta americana* (L.)] fed on a diet of whole wheat flour, skimmed milk powder, bakers yeast, and from 0 to 25 per cent *meso*-inositol were found by Srivastava (59) to be more resistant to lindane when the diet contained a maximum of 9 per cent *meso*-inositol.

Although there is this evidence which points to some effect of lindane in relationship to *meso*-inositol there are better reasons to doubt that the two materials have any specific physiological bearing upon one another. Metcalf (60) was unable to show any antidotal effects from the administration of *meso*-inositol to insects poisoned with lindane. Simultaneous injection of *meso*-inositol and lindane into *P. americana* by Dresden & Krijgsman (61) failed to antagonize poisoning by lindane and led them to question the validity of the hypothesis of Slade. Paulette (62) discusses the action of lindane on mammals, fish, molds, yeasts, bacteria, protozoa, and sea urchin eggs and concludes that lindane does not act as an antivitamin toward *meso*-inositol.

The geometric structure of lindane as proposed by Slade (53) would be designated as *peeeee*, indicating the positions of the chlorine atoms on each of the carbon atoms of the six-membered chair-form ring with respect to a vertical axis drawn through the plane of the ring. Chlorine atoms on bonds parallel to the axis are indicated as *p* and those equatorially disposed as *e*. The geometric structure of lindane determined by x-ray diffraction methods was found by Hassel (63) to be *pppeee* with respect to the position of the chlorine atoms on the ring. These findings were corroborated by van Volten *et al.* (64), and they concluded that lindane is not isomorphous with *meso*-inositol. Further confirmation is furnished by Lind *et al.* (65) who find that the dipole moment of lindane agrees with the theoretical calculated on the basis of the structure proposed by Hassel and by van Volten. The fact that the geometry of lindane is apparently different from that of *meso*-inositol now makes it seem that any competitive effects observed are from causes only indirectly related to the physiological effects caused by lindane.

Compounds which have such great differences in solubility as *meso*-

inositol, which is soluble in water, and lindane which is insoluble, could hardly follow similar pathways in insects, and it is difficult to conceive that they could interfere with one another to the extent of causing the evident physiological upset produced by lindane. The role of *meso*-inositol in the metabolic processes of an organism is not clearly understood, and this should be known before it is logical to implicate another compound as one which can induce poisoning through competition for a reactive site.

In the absence of any specific biochemical evidence to explain the mode of action of lindane, Mullins (21) proposes a physical basis which may account for the insecticidal action of lindane and the lack of insecticidal activity of the other isomers. He uses the same model of membrane employed to account for the insecticidal action of DDT, and finds that only lindane of all the known isomers of hexachlorocyclohexane can enter the membrane interspace in a plane orientation (Fig. 3). Using Stuart models Mullins determined that only lindane measures 8.5 Å (Table I) in all of the three diameters measured at intervals of 60° in the plane of the ring. All other isomers of hexachlorocyclohexane exceed 8.5 Å in one or more of the three measurements and are, thus, barred from entry in a plane orientation. All of the isomers can, however, enter the interspace in an end-on orientation.

Lindane entering the interspace in a plane orientation places the attractive forces of the halogen atoms in the most favorable position to act upon the surrounding lipoprotein molecules. If the attractive forces of lindane are strong enough to disturb the equilibrium position of the adjacent membrane

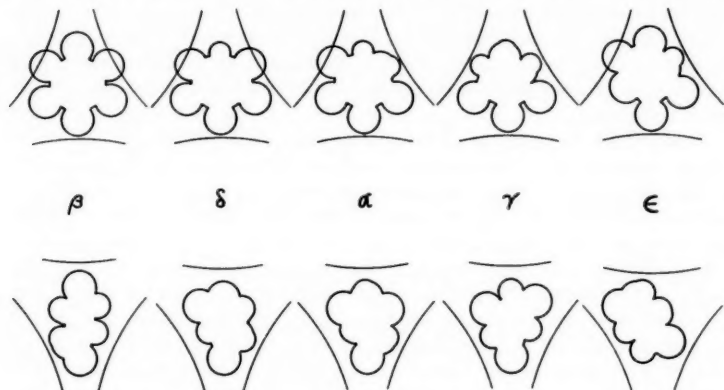


FIG. 3. Scale drawings from Stuart models showing the fit of various BHC isomers into a membrane interspace for plane and one end-on orientations. The interspace (shown by curved lines) is formed by membrane molecules of diameter 40 Å at a separation from each other of 2 Å. All plane orientations except that of lindane are excluded, while end-on orientations of all of the isomers are permitted. [From Mullins (21).]

TABLE I*
MOLECULAR CONFORMATION AND PHYSIOLOGICAL EFFECTS
OF THE ISOMERS OF BHC

Isomer	M.P. °C.	Con- figuration†	Molecular Diameters in Plane of Ring‡			Molecule Thickness	Physiological Effect
β	297	eeeeee	9.5	9.5	9.5	5.4	inert or weak depressant
δ	130	peeeee	8.5	9.5	9.5	6.3	strong depressant
α	157	ppeeee	8.5	8.5	9.5	7.2	weak excitant
γ	112	pppeee	8.5	8.5	8.5	7.2	strong excitant
ϵ	219	peeppee	7.5	9.5	9.5	7.2	not insecticidal
η	90	pepppee	7.5	9.5	8.5	7.2	not insecticidal
θ	124?	pepeeee	8.5	9.5	8.5	6.3	unknown

* From Mullins (21).

† The notation is such that *e* represents a chlorine atom located approximately in the plane of the cyclohexane ring; *p* represents chlorine atoms distributed alternately above and below the plane of the ring. [See Orloff, H. D., *Chem. Revs.*, 54, 347 (1954)].

‡ Insofar as the scale of Stuart models is accurate, these measurements can be considered in Å units. The three values for diameters in the plane of the ring are at intervals of 60° each.

molecules, distortion in the position of the molecules may lead to local regions of membrane instability and cause ion leaks which in the case of nerve cells would be manifest as excitation. The end-on orientation of any of the isomers into the membrane interspace can result only in narcosis which would be relative in magnitude to the thermodynamic activity of the particular isomer. Mullins points out that the thermodynamic activity of the different isomers can be approximated from their solubility in a paraffinic solvent. The order of solubility is $\delta > \gamma > \alpha > \beta$, with δ being about 100 times as soluble as β . This would account for the depressant effect observed for δ and might explain the antagonistic effect of α and δ isomers on the toxicity of lindane which van Asperen (78) reported in *Periplaneta*. An end-on orientation of any of the isomers does not provide a close fit for the molecule in the interspace, and the attractive forces of the halogens in this orientation could probably be involved with only one of the surrounding lipoprotein molecules, and this presumably would not lead to membrane distortion. Only lindane can enter the interspace in the end-on orientation and reorient into the plane.

Lindane versus DDT.—Aside from the fact that lindane and DDT are structurally quite different, there are other reasons to believe that their modes of action may be qualitatively as well as quantitatively different. Busvine (66) observed that houseflies poisoned with lindane "fan" their wings in an abnormal manner and this "fanning" is not seen in DDT poi-

soned flies. He quotes Barnes who observed that the widespread muscular twitching in rats poisoned with DDT is not seen with lindane, aldrin, dieldrin, endrin, and chlordane. More convincing of the fundamental differences in DDT and lindane is the fact that the development of resistance in houseflies to DDT by the selective action of DDT alone does not lead to a parallel increase in resistance to lindane, as pointed out by Busvine.

The effect of temperature on the insecticidal action of lindane has not been investigated as thoroughly as in the case of DDT, but data are available which show that temperature affects lindane poisoning much less than DDT. For example, Guthrie (67) found it required a 20-fold increase in DDT to produce an LD-50 in German roaches treated and held at 32°C. as compared to others held at 14.5°C. In a parallel experiment the difference in toxicity of lindane to roaches held at the two temperatures was only a factor of about two. In the case of aldrin and dieldrin higher temperatures favored rather than depressed their toxic action. Hoffman & Lindquist (45) had previously shown that with houseflies exposed to insecticide deposits DDT, methoxychlor, and TDE all had negative temperature coefficients. Hexachlorocyclohexane behaved the same at the two temperatures (70° and 90°F.) tested. Hoffman *et al.* (68) found that the sheep tick, *Melophagus ovinus* exposed to wool pads treated with different chlorinated hydrocarbon insecticides at temperatures of 70° and 90°F. behaved in a similar manner. They conclude that DDT, methoxychlor, and TDE are more effective at the lower temperature, whereas, hexachlorocyclohexane, toxaphene, and chlordane are more effective at higher temperatures.

The prevailing evidence on temperature effects points to a fundamental difference in the poisoning mechanisms of lindane and DDT. It also provides cause to question the hypothesis of Hurst (51) and its extension by Munson *et al.* (48) to explain the phenomenon purely on a physical basis, which is concerned with the holding capacity of lipides for the toxic agent. In addition to the simple measurement of the toxicity of compounds through a range of temperature, it would seem to be desirable to use the reversible effects of temperature as a criteria for characterizing the nature of a temperature coefficient of action. As previously pointed out, it can easily be demonstrated that the effects of DDT are reversible with temperature on houseflies, blowflies, roaches, and mosquitoes, but similar experiments with lindane, aldrin, chlordane, and dieldrin have indicated poisoning effects are not reversible with temperature changes [Rao (69)].

Lindane metabolism in houseflies.—The metabolism of lindane by insects other than houseflies has not been determined. It was shown by van Asperen & Openoorth (70) that lindane injected subcutaneously, intraperitoneally, or intravenously into white mice disappeared rather rapidly. One mg. of lindane applied subcutaneously to a mouse weighing 15 gm. was metabolized in four days, as shown by the analysis of tissue extracts subjected to the Schechter-Hornstein (71) test. Openoorth (72) injected lindane into susceptible and resistant strains of houseflies and followed the metabolism of

lindane at various subsequent intervals. The susceptible strain of flies metabolized lindane at a slow rate during the first 4 hr. and then stopped, which time may have been coincident with death. The resistant strains metabolized lindane at a rapid initial rate which decreased with time, but continued for 24 hr. when the total dose of 0.3 $\mu\text{g.}$ of lindane per fly was completely metabolized. Openoorth points out that the difference in the rate of metabolism of lindane by susceptible and resistant strains cannot be concluded to be the cause of resistance because the susceptible strain was killed by the dosage applied.

Openoorth (73) tested the metabolic breakdown of the alpha and delta isomers of hexachlorocyclohexane on lindane-resistant and susceptible strains of houseflies. The resistant strain in each case proved to have the greater capacity to metabolize these isomers. Since these isomers are nontoxic at the dosage levels used, Openoorth concludes that the greater capacity of the resistant strain to metabolize these compounds may be the cause for resistance to lindane, if it eventually can be shown that the metabolites are themselves nontoxic.

Bradbury *et al.* (74) by the use of C^{14} labeled lindane showed that a lindane-resistant strain of housefly was slower to pick up and absorb lindane from treated surfaces. They conclude, however, that this difference cannot account for the difference in level of resistance. In another experiment they assayed puparia and adults emerging from lindane treated media and found 40 $\mu\text{g.}$ of lindane per gm. of pupae and 17 $\mu\text{g.}$ per gm. of adults. From this they concluded that detoxication is not a factor in resistance since the flies contained much more lindane than would be required to kill a susceptible fly. This conclusion is not justified for several reasons. The assay method of Armstrong *et al.* (75), which they used, shows only that the responsive material in the extract of the fly will aromatize upon treatment with alkali and subsequently nitrate to produce a yellow product in the presence of sodium methylate. There are, theoretically, a number of nontoxic derivatives of lindane which could respond to this test. If the product found in the flies were lindane it would not rule out metabolism as a factor in resistance. It is conceivable that the insects consumed much more lindane in the course of their larval life than is indicated by the analysis, and that which is present may have gradually accumulated in some storage depot where it is incapable of functioning and unavailable to a detoxication mechanism.

More recent work of Bradbury & Standen (76) tend to confirm the previous findings of Openoorth, namely, that lindane-resistant flies metabolize lindane faster than susceptible strains. By exposing flies to residues of C^{14} labeled lindane for 6 hr. they find that both resistant and susceptible flies convert some of the absorbed lindane to a water soluble, carbon tetrachloride insoluble product. As much as 50 per cent of the lindane absorbed is metabolized by resistant flies. Since the nature of the water soluble metabolite is not known and the actual amounts of lindane taken up by the flies cannot accurately be determined by the methods they employed it is not possible

to draw conclusions regarding the significance of these findings with respect to lindane-resistance.

PYRETHRUM AND SYNERGISTS

Pyrethrum is a unique insecticide for a variety of reasons. It ordinarily will induce very rapid, temporary paralysis when applied in small dosages. The amount required to produce mortality is, however, usually much larger. Both its paralytic and lethal effects may be potentiated from 2 to as much as 12 times in mixture with certain compounds, generally termed "synergists," which may themselves have little or no measurable toxic properties. Pyrethrum appears not to produce harmful effects on mammals when administered dermally or orally in massive dosages. This is in sharp contrast to DDT and other chlorinated hydrocarbons where paralysis is relatively slow, ordinarily irreversible, effective synergists are not known, and toxicity to mammals somewhat parallels their toxicity to insects.

Site of action.—Because of its rapid rate of action, pyrethrum has generally been conceded to act in some way as a nerve poison. Lowenstein (79) proposed as a bioassay procedure the measurement of nerve action potentials induced by pyrethrum applied to the exposed central nerve cord of *Blatta orientalis*. The time required from the development of giant fiber potentials to extinction of all nervous activity was found to be inversely proportional to the amount of pyrethrum applied. Welsh & Gordon (14) compared DDT and pyrethrum applied to nerve muscle preparation of *Periplaneta*. They noted that the two materials produced similar spontaneous, rhythmic discharges in nerve trunks, but that pyrethrum acts much faster and in lower concentrations. The effects of pyrethrum were of shorter duration and could be reversed by perfusion with saline.

Kruger (80) studied the effects of pyrethrum poisoning on the transparent larvae of *Corethra*. He observed that within a short period of time, after the onset of convulsion, vacuoles appeared in nerve fibers. The vacuoles, however, disappeared a day later in larvae which survived. Histological studies by Hartzell & Wilcoxon (90) of nerve tissue from pyrethrum poisoned insects revealed a characteristic clumping of the chromatin of the nucleus of nerve cells and vacuolization in the ganglia and connectives of *Melanoplus* and *Tenebrio*. The degeneration of nerve tissue coincident with pyrethrum poisoning may not be considered as direct proof of the site of action and the result of pyrethrum poisoning for Richards (81) points out that similar effects may result from anaeroxia.

Evidence that pyrethrum attacks the nervous system seems indisputable, but conflicting opinions arise concerning the part of the nervous system which is attacked by pyrethrum. Roy *et al.* (82) concluded that the action of pyrethrum is confined to the central nervous system as opposed to the peripheral motor nerves. They found that pyrethrum applied to a thoracic ganglion of *Periplaneta* paralyzed the legs innervated by the ganglion. This

demonstrates the central effect, but does not exclude the possibility of pyrethrum attacking the sensory and motor nerves. Page & Blackith (83) calculated the distance pyrethrum theoretically could diffuse in the time interval between application and paralysis of adult *Aedes aegypti* (Linn.). They concluded that pyrethrum traveled no farther than the epicuticle, and, therefore, it must exert its effects very close to the surface of the insect, probably in the endings of the peripheral nervous system. While this may be true, it does not exclude the fact that pyrethrum can act upon the central nervous system. As pointed out by Earle (84) much of the controversy over the site of action could be resolved by making a distinction between the two degrees of intoxication (temporary paralysis of the appendages and death) and considering each one separately. The prevailing evidence indicates that pyrethrum may attack any area of the nervous systems.

The manifestation of typical poisoning symptoms may develop from pyrethrum applied to various parts of the body of an insect. This leads to the general belief that the material becomes uniformly distributed in the body of the insect. Earle (84) applied pyrethrum to the ventral cervical membrane of *Periplaneta*, and when the insects became moribund (28 hr. later) he tested the ventral nerve cord for nervous activity. He found response to cercal stimulation was normal in the abdominal portion of the nerve cord, but no evidence of nervous activity could be detected in the thoracic section. Similar experiments where pyrethrum was applied to the cercus caused typical poisoning symptoms. At the time the insects were moribund, cercal stimulation did not cause a nervous response, although there was a high level of spontaneous activity in the ventral nerve cord. Earle believes that paralysis results in this instance from the localized effect of pyrethrum on the cerci, causing incessant excitation of the ascending nerves which eventually "wears out" the system. The results of these experiments are good evidence that advanced poisoning effects may occur from localized action long before anything approaching general distribution of pyrethrum occurs.

Metabolic fate of pyrethrum.—The reversible paralysis induced by pyrethrum suggests that insects may possess a detoxication mechanism. Acree, Shaffer & Haller (85) believed hydrolytic enzymes such as esterases might attack pyrethrum and result in its decomposition to nontoxic products. Oral doses of pyrethrum administered to *Prodenia eridania* (Cramer) were found by Woke (86) to be detoxified in 6 to 12 hr. as determined by the bioassay of tissues and excrement. Woke also found that pyrethrum incubated with homogenates of blood, fat bodies, skin, muscle, and intestinal tract of this insect was decomposed in varying degrees, the fat bodies being the most efficient in this respect. Chamberlain (87) showed that lipase derived from *Periplaneta* would hydrolyze pyrethrum.

The chemical nature of pyrethrins immediately suggests hydrolysis of the esters as a logical process for *in vivo* detoxication, but there is good evidence to show that other decomposition mechanisms may be involved.

Winteringham (88) worked out a paper chromatographic scheme, whereby he could determine pyrethrins and their corresponding hydrolysis products. Using this method, Zeid *et al.* (89) were able to show that *Periplaneta* were capable of producing from pyrethrum the component acids and alcohols and a number of unidentified products. Winteringham (100) applied his technique to extracts of houseflies previously treated with pyrethrum and was able to find a product corresponding to the alcohol component obtained by hydrolysis of the esters, but he could not find evidence for the presence of the acid component. The presence of the product identified as the alcohol portion of the molecule favored survival in the flies. The absence of the characteristic acids may indicate that the esters may be changed in some way other than by hydrolysis to render them nontoxic.

Synergists.—When the combined action of two drugs administered at the same time produce an additive effect, as opposed to antagonistic, the summation is known as synergism [Goodman & Gilman (91)]. The term synergism as applied to pyrethrum has a slightly different connotation, because it applies to the potentiating effect obtained from a mixture of pyrethrum with certain compounds which are relatively nontoxic. The nontoxic compound is designated as the synergist. The first effective pyrethrum synergist, 1N-930 (isobutylundecylamide), discovered by Weed (92) has been replaced by the production of more effective synergists based upon the discovery of Haller *et al.* (93) that the methylenedioxyphenyl group was essential for the synergistic activity of sesamin. As a consequence, most of the synergists being used today are derivatives of isosafrole.

Since the mode of action of pyrethrum is not known, it is not surprising that the functions of synergists have not been adequately determined. Some significant findings have been made which may eventually lead to a better understanding of the mode of action of both pyrethrum and the synergists.

Page & Blackith (97) believed that a loose molecular complex is formed between the pyrethrum and synergist molecules which somehow increases toxic action. They arrived at this conclusion by finding that the reduction of the mean weight of pyrethrins required to paralyze *Aedes aegypti* (Linn.) by the addition of a synergist (sesamin or 1N-930) was proportional to the mole fraction of synergist until these were present in equimolar proportions with the pyrethrins. However, these findings do not agree with those of Gersdorf & Gertler (98) who report that increased activation may be obtained by increasing the molar ratio of synergist to pyrethrins to as high as 10:1. Further, and more direct evidence against the formation of a synergist-pyrethrin complex is furnished by the studies of Miller *et al.* (99), who found no evidence of a complex between piperonyl butoxide and pyrethrins as revealed by studies made on the infrared spectra and freezing points of mixtures of the materials.

Lindquist *et al.* (94) conducted a series of experiments on houseflies which revealed that flies pretreated with the synergist responded to subsequent

sublethal dosages of pyrethrum in much the same manner as if the two were applied simultaneously. When the reverse was attempted, they found that application of the synergist one, two, and four hr. after pyrethrum, failed to produce a synergistic action. Based upon these observations, the authors suggested that the synergist may cause slight injury or disarrangement of nerve tissue so that pyrethrum applied later is more effective in producing a knockdown. It is difficult, however, to conceive of a type of damage to nerve tissue which would not manifest itself in some usual expression of nervous disorder. Previous work of Hartzell & Scudder (95), which showed that the synergist 1N-930 in combination with pyrethrum caused chromatolysis of nerve cell nuclei, would offer support to the proposal of Lindquist *et al.*, if it could be shown that these histological observations are not merely nonspecific post-mortem effects.

The obvious question of the synergist acting as an agent to enhance the absorption of pyrethrum was considered and adequately answered by Wilson (96). He was able to demonstrate that about the same synergistic effect could be obtained by the application of pyrethrum and synergist to widely separated areas of the body of the housefly. From these experiments, Wilson concluded that the synergist did not act to enhance the penetration of pyrethrum through the insect cuticle, but the effect might be accounted for if it could be shown that the synergist inhibited some pyrethrum detoxifying system in the insect.

Chamberlain (87) confirmed the findings of Wilson. He also found that flies were more sensitive to pyrethrum at low temperatures, but in mixture with the synergist piperonyl butoxide [(butyl carbitol) (6-propylpiperonyl) ether] the temperature-sensitivity disappeared. An explanation of these findings based upon a pyrethrum detoxication mechanism, would imply that flies are less sensitive to pyrethrum at high temperature because the detoxication mechanism is accordingly activated and that inhibition of the detoxifying mechanism by the synergist is correspondingly more efficient at higher temperatures. These speculations are consistent with other proven relationships between enzymes, substrates, temperature, and inhibitors, and there are presently no reasons to discard this as a possible explanation of the mode of action of pyrethrum synergists.

Chamberlain extended his studies to consider the effect of lipase derived from *Periplaneta* on pyrethrum and the effect of synergists upon hydrolysis of pyrethrum. Under the particular conditions of this experiment, the pyrethrins underwent enzymatic hydrolysis, and this could be inhibited to varying degrees by the addition of different pyrethrum synergists. The correlation between the lipase-inhibiting properties of the synergists did not correspond with their proficiency as pyrethrum synergists. This might be expected in view of the previously mentioned findings of Winteringham (100) and Zeid *et al.* (89) whose work would suggest the existence of mechanisms in addition to hydrolysis as responsible for the detoxication of pyrethrum. The as-

sumption that synergists may exert their effect by inhibiting a pyrethrum detoxifying mechanism still is the most attractive explanation of their mode of action even though direct proof has not been furnished.

ORGANOPHOSPHATES

Previous to the studies on the mode of action of the organophosphates on insects, it had already been established that certain of these compounds were potent inhibitors of the cholinesterases (ChE's) of mammals [Adrian, Feldberg & Kilby (102); Webb (103)]. The presence and indispensability of ChE's in "the conductive tissues of all members of the animal kingdom" [Nachmansohn & Wilson (104)] would predict similar effects for these compounds on insect ChE's. This was found to be true by Chadwick & Hill (105), Dubois & Mangun (106), Metcalf & March (107) and others, and in general, a good correlation was found between the degree of inhibition and mortality. If one accepts the general concept of neural transmission involving acetylcholine (ACh) as a "chemical mediator," then the inhibitory effects of the organophosphates on ChE's would seem to be a satisfactory explanation of their mode of action on insects.

Inhibition of noncholinesterases.—Such a conclusion does not imply that the organophosphates are not involved as inhibitors and substrates for other enzyme systems in insects which may also be vital to the organism. As a matter of fact it has been shown that certain of the organophosphates will inhibit chymotrypsin [Balls & Jansen (108)] and other less well defined enzymes [Lord & Potter (109); Hopf (110)]. It has also been shown that they may be changed *in vivo* from noninhibitors to powerful anti-ChE's as illustrated by conversion of parathion to an active anti-ChE, probably para-oxon [Gage (111); Kok & Walsh (112)] and octamethylpyrophosphoramide to the phosphoramidate oxide [Cassida *et al.* (113)]. It is quite likely that substantial proportions of any of the organophosphates are rendered inactive by enzyme attack *in vivo* before they ever have an opportunity to inhibit ChE's. Aldridge (137) has shown that para-oxon may be hydrolyzed enzymatically by liver slices to produce noninhibiting products. If it is true that the mechanism of ChE inhibition by the organophosphates is on a mole for mole basis as proposed by Burgen (114), Brauer & Pessotti (115), Bournsnel & Webb (116), and Jansen *et al.* (117), then obviously a great percentage of the dosage of organophosphates required to kill an organism has a fate other than inhibition of ChE's.

If it could be shown that any of various *in vivo* pathways of the organophosphates involved the effective inhibition of a system equally as vital as the ChE's, then this would have to be considered as an additional cause of poisoning. Lord & Potter (109) have demonstrated the presence of an esterase in four different species of insects which is capable of hydrolyzing phenylacetates. Furthermore, they find this system to be distinct from ChE's although it was inhibited by all of five organophosphates which they tested. The concentration of inhibitor required was found to be of the order of mag-

nitude as required to inhibit ChE's from the same species of insects. It is logical to assume that the presence of a phenylesterase has a role in the metabolic processes of these insects or it would not be present, and since it is inhibited by the organophosphates, it cannot be disregarded in assessing the over-all poisoning process induced by the organophosphates. It now becomes necessary to determine the vital role of this enzyme system in insects in order to evaluate its importance relative to ChE's.

Hopf (110) studied the inhibition of "acetylsterases" from the nerve cord of *Locusta migratoria migratorides* Reiche and Fairmaire and horse blood serum. He found homogenates prepared from nerve cord were capable of hydrolyzing both ACh and *o*-nitrophenyl-acetate (NPA), and that the enzymes responsible for their hydrolysis were equally inhibited by the same concentrations of tetraethylpyrophosphates. From this he suggests that the two substrates may be subject to attack from a nonspecific esterase. Hopf (110) did not, however, carry out his experiments in such a way as to exclude the existence of "acetylcholinesterase" [see Nachmansohn & Wilson (104)]. If Hopf was working with the same phenylesterase studied by Potter & Lord (109), then one may use the latter's findings to conclude that a phenylesterase system may be separated from an ACh splitting mechanism. Hopf's studies are important because they associate the NPA esterase with the nervous system and show that *in vivo* inhibition of the enzymes involved in the hydrolysis of ACh and NPA are equally inhibited at 4 hr. in locusts injected with a series of organophosphates.

There is an obvious need for further studies on enzyme systems such as those reported by Lord & Potter and Hopf in relation to the organophosphates. There can be no doubt that they have a role to play in the poisoning process. It is necessary that they be better defined with respect to their function in the normal physiology of the organism, and a great deal more must be determined about the kinetics of hydrolysis of various substrates which they may attack before their over-all significance can be determined. It must be borne in mind that these enzyme systems may be part of a variety of esterases which may constitute an important part of the defense mechanism of the insect against the organophosphates.

ChE system in insects.—Lord & Potter (118, 119) questioned the presence of ChE as a vital system common to all insects because they were unable to demonstrate its presence in the larvae of *Tenebrio molitor* Linn. and adult *Tribolium confusum* (Hbst.). It has since been shown by O'Brien (120) that the procedure used by Lord & Potter for measuring ChE in these insects were such that the enzyme would have been inactivated or discarded as a centrifugate. O'Brien used standard procedures for the preparation of homogenates and was able to demonstrate the presence of active ChE in the same species. Apparently Potter & Lord (109) have since satisfied themselves of the presence of ChE in these species for they report experiments demonstrating enzymatic hydrolysis of ACh in more recent studies.

Other workers have reported findings which in one way or another indi-

cated the ChE's or the chemical mediator might be different in insects from those in mammals. Richards & Cutkomp (9) reported the ChE's from honey bees and roach nerve cord hydrolyzed acetyl- β -methylcholine about three times as fast as ACh, which would suggest that ChE of insects was different from that of vertebrates and possibly that ACh was not involved as a mediator in insects. These workers could not have known it at the time, but the apparent difference was attributable to their use of ACh concentrations above the optimum which have an inhibitory effect upon ChE as emphasized by Augustinsson (122). It is apparent that as more insects and organophosphates are studied with respect to ChE, discrepancies in results may be expected. To avoid gross misinterpretations, it is suggested that workers refer to the criteria established by Nachmansohn & Wilson (104) for the characterization of ChE and modify the usual procedures for preparation of vertebrate ChE to conform to the latest findings of Chadwick, Lovell & Enger (123, 124) with respect to the reaction conditions which provide the maximum reaction rate with ChE's derived from insects.

Hopf (125) suggests that the "physiology of the nervous system of the insect is different from that of the mammal, neither cholinesters nor adrenalin being concerned in it." His suggestion is supported by experimental evidence which deserves serious consideration. Roeder & Roeder (126) were able to demonstrate the effect of anti-ChE drugs on nerve transmission in *Periplaneta* but could not explain their tolerance for high concentrations of ACh. Tobias, Kollros & Savit (8) found with *Periplaneta* that injected dosages of 7 to 10 gm./kg. of acetylcholine and 1 gm./kg. of carbachol were required to produce intoxication, and that roaches would tolerate as much as 20 gm./kg. of acetyl- β -methylcholine without effect. Since the ultimate toxic effect of ChE-inhibition is presumably a result of accumulation of quantities of ACh above threshold, Hopf believes the amounts of ACh required for poisoning are in great excess of those which could be released or synthesized by the insect after the inhibition of the ChE's. To further emphasize the difference in response of vertebrates and insects, with regard to cholinesters, Hopf cites LD-50's from Goodman & Gillman (91) for subcutaneous application of cholinesters to white rats as follows: ACh, 250 mg./kg., carbachol 4 mg./kg. and acetyl- β -methylcholine 750 mg./kg. The difference in sensitivity between the roach and rat is obvious and a satisfactory explanation is not available.

Further work of Hopf (110) on antagonists and potentiators of ACh provide more evidence to dispute the assumed role of ACh and ChE's in insects. Using the migratory locust as a test animal, he found it impossible to demonstrate an antagonistic effect for atropine against tetraethylpyrophosphate or eserine when applied as injected doses and in a wide range of concentrations. Since atropine abolishes the muscarinic effects of ACh in mammals and is used as an antidote for poisoning by ChE-inhibitors, its lack of similar effects on insects is unexplainable. In other experiments Hopf shows that potentiation of poisoning by tetraethylpyrophosphate cannot be accom-

plished with large doses of ACh. The locust is insensitive to tubocurarine and neostigmine (Prostigmine) but is sensitive to eserine. All of this adds up to the inescapable conclusion that the neurophysiology of insects may not be understood by extrapolation of information gained from studies on vertebrates.

To explain the observations of Hopf and others and defend the classical interpretation of nervous function based on acetylcholine as an indispensable chemical mediator would be a task which would have to be accomplished almost entirely on a speculative basis and would probably serve no useful purpose. Some evidence which may have a bearing on the problem are the findings by Nachmansohn *et al.* (127) that about 50 per cent of the pharmacological activity obtained by the *in vitro* synthesis of ACh with rabbit brain cholineacetylase is not actually attributable to ACh. The unknown agent is not a derivative of choline since the product is produced in the absence of choline. Fernando (128) found that extracts prepared from nerve cords of *Periplaneta* contained a product which behaved pharmacologically like ACh but did not respond to the Hestrin (129) test. This led him to conclude that the ACh-like substance in the nerve tissue could not be accounted for entirely as ACh even though the material is readily inactivated by nerve cord homogenate. When these unidentified products are better understood, we may have an explanation for some of the differences observed between vertebrate and insect neurophysiology.

There appears to be no reason to question the presence of a material in the nervous tissue of insects which behaves pharmacologically like ACh. Corteggiani & Serfaty (130) found the equivalent of 100 to 200 $\mu\text{g./gm.}$ in the nerve cord of *Tenebrio molitor* Linn., 65 $\mu\text{g./gm.}$ in the brain of *Gryllus domesticus* Linn., and 200 $\mu\text{g./gm.}$ in *Xylocopa violacea* (Linn.). Mikalonis & Brown (131) found the nerve cord of *Periplaneta* contained an ACh equivalent of 70 $\mu\text{g.}$ bound and 40 to 200 $\mu\text{g./gm.}$ free. Only recently, however, has more specific evidence been presented to indicate the presence of ACh in insects. Lewis (132) found that extracts of *Calliophora erythrocephala* (Meig.) and *Lucilia serricata* Meig. contained ACh-like compounds which chromatographically were identical with ACh chloride. By a different chromatographic procedure Augustinsson & Grahn (133) found evidence of the presence of ACh in extracts from the head of honey bees, and more recently Chefurka & Smallman (134) were able to chromatographically confirm the presence of ACh in extracts of house flyheads. This better evidence of the presence of ACh in nervous tissue of insects helps answer the question of its existence in insects, but does not aid in refuting the questions raised by Hopf and Lord & Potter in regard to its vital role in the nervous activity of insects.

Insect versus vertebrate ChE.—It is not yet practical to make comparative studies on ChE from various tissues and animals with highly purified preparations. Consequently, tissue homogenates are almost invariably used as a source of ChE. The presence and activity of ChE under these conditions is determined by the usual criteria of substrate specificity, effect of inhibitors,

and reaction kinetics. A large and variable amount of extraneous materials which may influence the course of ACh hydrolysis must be anticipated in comparing ChE activity from one tissue to another and more so from one organism to another. This makes it difficult to know whether tests which seem to show a qualitative or quantitative difference in ChE are actually true or whether it is merely the effect of unknown components in the reaction mixture. Certainly under these conditions, small differences should not be considered significant and indicative of a modified enzyme system.

Metcalfe & March (135) compared the ChE levels in honey bee, housefly, and mouse brains and found differences of such magnitude that they cannot be disregarded. Under the conditions of their experiment, the micromoles of ACh hydrolyzed per mg. of brain in 30 min. were 0.356, 2.5, and 11 for mouse, bee, and fly brain respectively. The authors point out that fly brain is one of the richest sources of ChE known, being in the class of the electric organs of *Torpedo* sp. and *Electrophorus electricus* in this respect. The difference in ChE levels of the three species does not in itself reflect a qualitative difference in ChE. The substrate activity curves for the three sources of ChE were characteristically bell-shaped with concentration optima at 10^{-2} M ACh, which is considered to be good evidence of identity. Differences were found in respect to hydrolysis of other choline esters. Acetyl- β -methylcholine was hydrolyzed rapidly by bee brain and only slightly by housefly brain ChE. One of the most significant findings of Metcalfe & March was the fact that 0,0-diisopropyl-*p*-nitrophenyl thiophosphate was almost inactive against bee brain ChE but highly inhibitory against fly brain ChE, the difference here being a matter of about 1000-fold. This correlates with the fact that 0,0-diisopropylthiophosphate is practically nontoxic to bees and highly toxic to houseflies.

The result of these experiments led Metcalfe & March to conclude that distinct biochemical differences exist in the specific cholinesterase from the brains of the honey bee, housefly, and white mouse. Another explanation for the specific activity of 0,0-diisopropyl-*p*-nitrophenyl thiophosphate might now be proposed on the basis of the gradually accumulating evidence that the thionophosphates [$\equiv P \rightarrow S$] must be converted to the corresponding phosphates [$\equiv P = O$] in order to become ChE inhibitors *in vivo* [Gage (111), Myers *et al.* (136), Kok & Walsh (112)]. The alternative explanation would imply that the honey bee does not have the mechanism to effectively convert the specific compound to the phosphate, whereas, the housefly does. One would have to further postulate that the housefly can carry out the conversion both *in vitro* and *in vivo*. This would be somewhat unexpected, because pure parathion ordinarily does not readily inhibit ChE *in vitro* but does *in vivo*. The important findings of Metcalfe & March have probably been instrumental in focusing the attention of investigators on the necessity of considering the fate of organophosphates both *in vivo* and *in vitro* in relation to ChE-inhibition.

The fact that many organophosphates inhibit chymotrypsin [Balls &

Jansen (108)], which may be obtained in pure form, may serve as a valuable tool to distinguish those compounds which may directly inhibit ChE from those which must undergo metabolic changes before they attain an active state. This approach has already been used by Cassida, Allen & Stahmann (113) to demonstrate that octamethylpyrophosphoramidate must be metabolically or otherwise oxidized before it can become an inhibitor of either ChE or chymotrypsin.

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THE CHEMISTRY OF INSECTICIDES^{1,2}

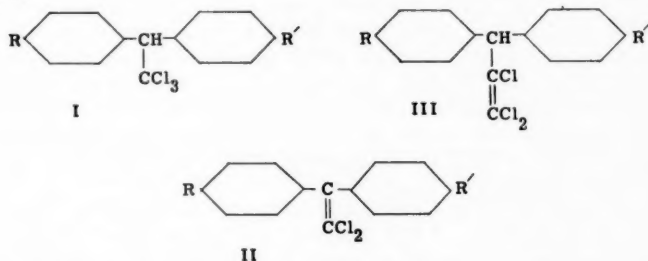
BY HUBERT MARTIN

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DDT AND ITS ANALOGUES

The success of DDT as an insecticide at once stimulated the examination of analogous compounds, and it became evident that high insecticidal activity was shared by but a few of these derivatives. This situation immediately led to speculation on the reasons why insecticidal activity should be so limited and on the features of the DDT molecule which rendered it highly toxic to insects. Despite the handicap that positive information on the biological reactions of DDT have even yet not been derived from biochemical and biological studies of DDT, investigations prompted by the chemical hypotheses have given clues to the nature of the insecticidal action of DDT and have exposed to some degree the molecular structure requisite for insecticidal activity in this series of compounds.

An active insecticide must have the physical properties requisite for its entry to, and accumulation at the responsive sites within the insect. It is, therefore, not surprising that DDT analogues of the type (I) in which R (= R') is a grouping such as hydroxyl, sulfo, carbonyl or nitro, polar enough to reduce lipid solubility, should have negligible insecticidal activity. But this simple hypothesis is inadequate to explain, in the dialkyl and dialkoxy derivatives, the reduction of activity which occurs when R (= R') is greater than ethyl or ethoxy, or why activity is absent in those DDT-like compounds in which the phenyl groups are replaced by naphthyl groups. Nor can considerations of lipid solubility or of surface activity account for the reduction in the *o-p'* series (I, R in *o*-position), its absence in the *o-o'* series (I, R and R' in *o*-positions), or why the ethylenes (R·C₆H₄)₂C:CCl₂ (II) are non-toxic.



¹ The survey of the literature pertaining to this review was completed in June, 1955.

² Contribution No. 50, Science Service Laboratory, Canada Department of Agriculture, University Sub Post Office, London, Ontario, Canada.

The differences in the insecticidal activities of *p,p'*-, *o,p'*- and *o,o'*-DDT are a challenging problem and have directed attention to those hypotheses which relate insecticidal activity to molecular configuration rather than to those which relate activity to the presence of specific toxophore groupings. A recent example of the former type of argument is attributable to Riemschneider & Otto (1) who patterned molecular structure on Stuart models. In 1,1-diphenyl-2,2,2-trichloroethane a free rotation of the two phenyl groups and of the trichloromethyl group is restricted only by the slight repulsion of the *o*-hydrogens of the phenyl groups, and this condition is not affected by *p,p'*-substitution. If, however, the *o*-position is occupied by a group as bulky as the chlorine atom, the rotation of the phenyl groups becomes impossible and, indeed, two isomers, not counting stereoisomers, can exist in *o,o'*-DDT of which the more stable is that in which the two *o*-chlorine atoms are on opposite planes of the phenyl rings. Similarly, in the symmetrical tetramethyl analogues examined by Müller (2) the 2,2', 4,4'- and the 2,2', 5,5'-isomers showed little insecticidal activity whereas the 3,3', 4,4'-isomer, in which rotation of the phenyl rings is possible, showed insecticidal activity. It will be recalled that Wild (3) deduced from dipole data that, in 2,2', 4,4'-tetramethyldiphenyltrichloroethane, free rotation of the phenyl groups is not possible.

The activity of the DDT isomer, 1,1-bis(*p*-chlorophenyl)-1,2,2-trichloroethane, reported by Müller (2) to be as insecticidal as DDT to houseflies, but now accepted as generally of lower potency, was contrasted by Riemschneider & Otto (1) with the inactivity of the corresponding 1,2,2-tetrachloro-derivative; in the former, the rotation of the phenyl groups is permitted by a to-and-fro manipulation of the dichloromethyl group; in the latter, rotation is impossible.

Riemschneider & Otto (1) extended their hypothesis to the DDD analogues, but in such compounds the smaller bulk of the dichloromethyl group in relation to the trichloromethyl group permits rotation of the phenyl groups even when the latter are substituted in the *o*-position by chlorine or methyl groups. Yet, generally speaking, this series of compounds have insecticidal activities well below those of the analogous DDT compounds though there are notable exceptions against specific insects which have been put to practical use. Examples are the recent introduction of 1,1-bis(*p*-ethylphenyl)-2,2-dichloroethane and the older use of DDD for the control of *Eulia valutinana* and *Tortrix citrana*. The resistance of the larvae of both of these tortricids to DDT would suggest that DDD here differs from DDT in its interference with vital processes.

More convincing is the evidence given by Riemschneider & Otto (1) on the 2,3,3-trichloro-1,1-diphenylpropylenes (III). In these compounds rotation of the phenyl groups is not hindered by the trichloropropylene group even when the *o*-positions are occupied by chlorine or methyl. Consequently, the tetramethyl compounds exhibit insecticidal properties, as shown by Müller (2), irrespective of whether the *o*-positions are occupied or

not. In the series of compounds examined by Müller, which also include the compounds in which $R=R'=H, Cl, F, Br, -CH_3$, and $-OCH_3$, insecticidal properties were of a lower order than in the corresponding DDT analogues.

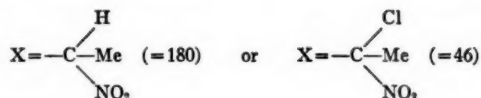
Riemschneider & Otto stated that in the ethylene series (II) a free rotation of the benzene rings is completely inhibited; none of these compounds has insecticidal activity. Moreover, the lack of insecticidal properties in the naphthyl derivatives can be explained because of the *o*-position is here occupied by a bulky grouping. The least satisfactory of the correlations deduced by Riemschneider & Otto is that which concerns the positional isomers of DDT. They claimed a complete parallelism between the freedom of rotation of the phenyl groupings and the insecticidal activity of the isomers, namely, $p,p'- > m,p'- > o,p'- > m,o'- > o,o'$ -DDT. Yet from dipole moments Wild (3) showed that free rotation is impossible in *o,p'*-DDT, a compound which has insecticidal activity not greatly below that of 1-(*p*-chlorophenyl)-1-phenyl-2,2,2-trichloroethane [see Tables 19 and 20, Metcalf (4)], and in this compound rotation is not restricted.

An alternative structural hypothesis is attributable to Brown *et al.* (5). They pointed out that in a compound having a carbon atom linked to two or three planar groups and a group which, though capable of rotation itself, is large enough to hinder the rotation of the planar groups, then the latter will tend to positions of maximum clearance, that is, to positions corresponding to the sides of a trihedral angle. Such a condition exists in 1,1-diphenyl-2,2-dimethyl propane (a DDT analogue in which the trichloromethyl group is replaced by a *t*-butyl group). Brown *et al.* suggest that, though in DDT itself, a rotation of the phenyl groups is possible, strain compels the phenyl groups to assume a trihedralized configuration. To test their hypothesis, Brown *et al.* examined the insecticidal activity of a series of DDT-like compounds in which the trichloromethyl group was replaced by groups of similar or larger molecular dimensions; they were thus led to the discovery that the chlorine-free analogue, 1,1-dianisyl neopentane, produces in insects the tremors characteristic of DDT. Support for this hypothesis is given by the previously-discovered insecticidal activities of 2-nitro-1,1-bis(*p*-chlorophenyl) propane and the corresponding butane, for in these DDT analogues the trichloromethyl group is replaced by nitro alkyl groups [$-CH(NO_2)CH_3$ and $-CH(NO_2)C_2H_5$ respectively] of equal or greater bulk. Moreover, the homologous ethane and pentane derivatives have negligible insecticidal activity.

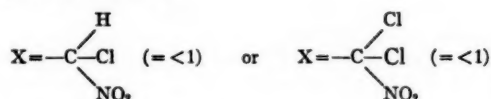
Difficulty is, however, encountered in the application of the trihedralization hypothesis to the results of Woodcock & Skerrett [see Woodcock (6)] on the DDT isosteres. These authors applied the well-known device of replacing the substituent groupings of a compound of known biological activity by groups of similar molecular dimensions. For example, the Van der Waal radii of the hydroxyl (1.7 Å) and methyl (2.0 Å) groups are close to that of the chlorine atom (1.8 Å). The hydroxyl group, however, is an unsatisfactory substitute for the chlorine atom since its presence would greatly modify con-

ductophoric properties such as solubility. Consequently, Woodcock & Skerrett sought to replace the chlorine atoms of DDT by methyl groups. The replacement of the trichloromethyl group by $-\text{CClMe}_2$ removed insecticidal properties to *Calandra granaria*, yet trihedralization is present in the resultant compound, 1,1-bis(*p*-chlorophenyl)-2-chloro-2-methyl propane.

Moreover, in the nitroalkane series (*p*-Cl·C₆H₄)₂CHX synthesized by Skerrett & Woodcock (7) and tested on *C. granaria*, insecticidal activity was present when



yet the compounds in which

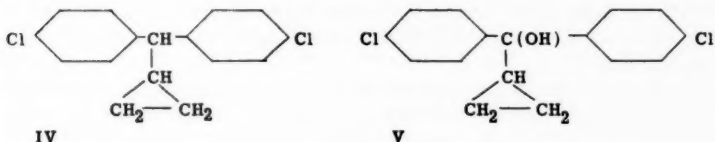


were comparatively harmless to these insects. The figures in parentheses refer to the potency of the compound relative to that of DDT (=100). A final word on this dilemma should await the outcome of tests on a wider range of insects for *C. granaria* is but little affected by 1,1-dianisyl neopentane (<1), a compound found toxic to many other insects. There is here revealed the sore need for *in vitro* tests, whether enzymological, surface-active, or electrophysiological, which will yield a measure of activity free from the effects of the conductophoric factors influencing penetration and transportation to the site of insecticidal action.

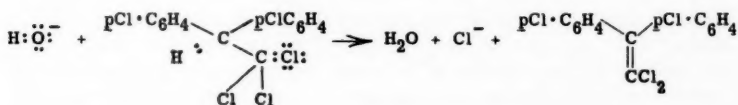
Subsequent to writing the above paragraph the reviewer was privileged to receive the manuscript of a paper by Stringer, Woodcock & Skerrett (8) in which are recorded the results of further tests of their DDT isosteres. He is indebted to the authors for permission to quote, not only the above bracketed figures of relative potency to *C. granaria*, but the following figures for potency to adult male cotton stainers (*Dysdercus fasciatus*): DDT=100, methoxychlor=186, 1,1-dianisyl neopentane=25. The authors attribute the comparative nontoxicity of many analogues of DDT which might be expected to exhibit insecticidal activity to the operation of biological factors preventing a concentration of the compound at the site of action.

The two structural hypotheses so far considered deal with spacial distribution, a consideration which has a bearing on older hypotheses. For example, Lauser, Martin & Muller (9) in their first account of the insecticidal properties of DDT associated that property with the lipophilic character of the trichloromethyl group. Accordingly, Biro, Voegtli & Lauser (10) examined the cyclopropane analogues. Bis(*p*-chlorophenyl)cyclopropyl methane (IV) proved about four times less toxic than DDT to houseflies yet di-(*p*-chloro-

phenyl)cyclopropyl methanol (V) was as toxic as DDT. Biro and his co-workers suggested that the greater potency of the methanol derivative (V) is a result of the hydroxyl group which hinders the free rotation of the phenyl groups, a hindrance demonstrated by the low reactivity of the hydroxyl group in V, which forms neither a urethane nor a phenylurethane.



Early in the history of DDT, Martin & Wain (11) pointed out that, of the limited series of DDT analogues they tested, only those which are readily dehydrochlorinated by alcoholic alkali are toxic to insects. The mechanism of this dehydrochlorination reaction is generally regarded as an E_2 elimination represented by:



Conformity to this equation was shown in the kinetic studies of Cristol and his colleagues (12) of a series of *p,p'*-substituted diphenyl-trichloroethanes, dichloroethanes, and monochloroethanes. In all these compounds rotation of the phenyl group is unrestricted and the rate of dehydrochlorination in each series is determined by the effect of the *p*-substituents on the strength of the $-C-H$ bond. But if rotation is inhibited or if the hydrogen atom is sheltered by the substituent on the phenyl groups, this simple process is no longer possible. Although little is as yet known of the mechanism and kinetics of the restricted case, the reviewer cannot resist the suggestion that there is a common basis to Martin & Wain's observation on the relationship between insecticidal properties and ease of dehydrochlorination and to the hypotheses linking steric hindrance and insecticidal activity.

Although there seems to be a conflict between the hypothesis advanced by Riemschneider & Otto and that of Brown and his colleagues, some reconciliation is possible on the following reasoning: Fischer's "lock and key" simile of specificity has been put into practical terms by Pauling (13) who invoked the attractive forces which become significant only when the active groups are brought into the closest proximity. If to be active the toxicant molecule has to become attached to a sensitive interface such as a protein of an enzyme or a nerve membrane (using this term without cyto-geographical implications) then it must be of such a shape that it can fit closely and

snugly along that interface. It must, in other words, be capable of assuming a spacial configuration which is complimentary to that of its site of combination. The hypothesis of Brown and his colleagues suggests that the required fit is provided by the trihedral configuration; that of Riemschneider & Otto requires that the molecule shall be able to assume that configuration. If now the latter hypothesis is extended to include as toxic those DDT analogues in which rotation is restricted yet which in the restricted state have that special configuration, the apparent conflict between the two hypotheses becomes less important.

In a preliminary paper, Gunther *et al.* (14) have applied, with Pauling's help, the ideas of the last paragraph to a quantitative examination of the insecticidal activities of the series of DDT analogues, homomorphs, of diphenylethane, in which either or both of the nuclear chlorines of DDT or one or more of the three aliphatic chlorines are replaced by hydrogen or methyl groups. The toxicities of these compounds when expressed as negative log LD_{50} are directly proportional to the sum of the logarithms of the Van der Waal's attractive forces of the five substituent groups. They point out that this hypothesis, which they name the structural topography theory, concerns only the primary action, the interaction by which the DDT-like molecule becomes attached to the protein surface; it provides no information on the biological consequences of the formation of the DDT-protein complex. For the primary process, the size of the group whether on aromatic or aliphatic carbon is of equal significance. Interaction is weak when there are too many hydrogens in these positions, an indication that the fit of the unsubstituted compound to the protein surface is poor.

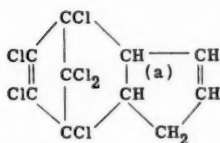
In a later paper read before the American Chemical Society at Cincinnati in April, 1955, Gunther, Blinn and Carman developed their ideas on the conditions requisite for the interlocking of enzyme and DDT-like insecticide. A distinction is made between those forces which involve "fit" (complementariness) and those associated with "hold" (polarizability). "Fit" is determined by Van der Waals radii and "hold" is determined by Van der Waals attractive forces. They extended these experimental tests to the homologues of diphenyl methane having found that these compounds, though not very toxic to mosquito larvae, were highly toxic to the confused flour beetle, *Tribolium confusum* Dew. High variance in the bioassay unfortunately frustrated the use of these results to test their hypothesis.

The conception that the toxic DDT analogue must have the configuration and dimensions permitting a snug fit at some interface within the insect explains the earlier observations of Busvine (15), of Prill, Synerholm & Hartzell (16), and of Picard & Kearns (17). The first author concluded that the most toxic analogues of DDT are those most similar to the DDT molecule in shape and weight; the second and third suggested that, for high insecticidal activity, substitution must be in the *p,p'*-positions and that the substituents should not be larger than a certain critical size. These latter qualifications have been given greater meaning by the various hypotheses of free rotation,

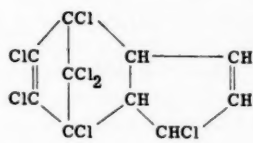
of trihedralization and of steric hindrance and, particularly, of structural topography which were earlier discussed in this section.

THE HEXACHLOROCYCLOPENTADIENE GROUP

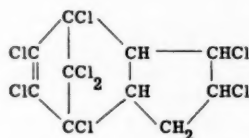
Hexachlorocyclopentadiene is the intermediate of a remarkable group of insecticidal compounds of which the chemistry, published mainly in the patent literature, is still but poorly known in many of those aspects which are associated with insecticidal properties. Chlordene ($C_{10}H_6Cl_6$, 4,7-methano-3a,4,7,7a-tetrahydro-4,5,6,7,8,8-hexachloroindene) (VI) which is the Diels-Alder condensation product of hexachlorocyclopentadiene with cyclopentadiene, is but feebly insecticidal, but this activity is greatly enhanced by controlled chlorination. Under certain conditions, e.g., in benzene with chlorine



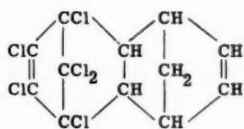
VI



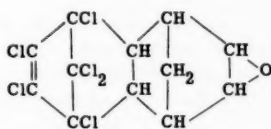
VII



VIII



IX

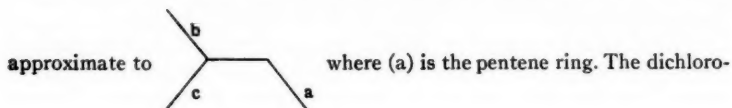


X

in the dark and in the presence of Fuller's Earth (U.S. Patent 2,576,666) the main product is heptachlor ($C_{20}H_6Cl_7$, 4,7-methano-3a,4,7,7a-tetrahydro-1,4,5,6,7,8,8-heptachloroindene) (VII). Under other conditions, e.g., by sulfuric chloride in the presence of a Friedel-Craft catalyst such as aluminium chloride (U.S. Patent 2,508,561), the main product is chlordane ($C_{10}H_6Cl_8$, 4,7-methano-3a,4,7,7a-tetrahydro-2,3,4,5,6,7,8,8-octachloroindane) (VIII).

In all three compounds, *endo-exo* isomerization is possible. March (18) concluded that only one, now thought to be the *endo* configuration, was pres-

ent. This type of isomerization arises because the carbon skeleton of the chlordene molecule does not lie in one plane; if viewed edge on, it would



methano bridge may lie in position (b) or (c), the former being the *endo* isomer and the latter the *exo* isomer. In addition to this type of isomerization, chlordane is capable of *cis-trans* isomerization and, indeed, two isomers α - and β -chlordane have been differentiated, the α -isomer melting at 104.5–6°C. and the β -isomer melting at 106.5–8°C. Because the β -isomer is less readily dehydrochlorinated by alcoholic potash than the α -form, it is thought that the β -form is the *trans* isomer.

The Diels-Alder condensation of hexachlorocyclopentadiene with vinyl chloride and the subsequent dehydrochlorination of the adduct yields 1,2,3,4,7,7-hexachlorobicyclo[2,2,1]-2,5-heptadiene which is perhaps an intermediate of a group of insecticides of which aldrin, dieldrin, endrin, and isodrin have so far been introduced. Either aldrin or endrin may be the Diels-Alder adduct of this compound with cyclopentadiene for these insecticides are the *endo-exo* and *endo-endo* isomers respectively of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1:4,5:8-dimethanonaphthalene (IX). Particulars of the manufacturing processes of aldrin are not available, but it would appear that the Strauss process was replaced in 1952 by the Thermal process in order to avoid the presence of a highly poisonous compound in the Strauss product [Ball *et al.* (19)]. The situation is perhaps parallel to that attributable to the high vapour toxicity of early samples of chlordane which was shown by Ingle (20) to be a result of unreacted hexachlorocyclopentadiene, the presence of which is reduced to a minimum in the more recent manufactured product.

Whereas, in these compounds, the —CCl=CCl— group is stable and unreactive, the —CH=CH— group may be epoxidised, the resulting compounds (X) from aldrin and isodrin being dieldrin and endrin respectively. The epoxidation reaction may be of significance in the biological reactions of aldrin and dieldrin for Davidow & Radomski (21) isolated, from the fat of dogs fed with heptachlor, a compound which likewise is susceptible to epoxidation, a metabolic product identified as an epoxide of heptachlor.

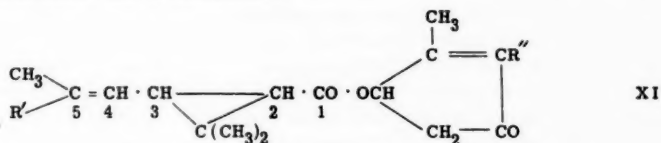
Unhappily, little is yet known of the biological interactions of this group of compounds, nor of the vital processes which are involved. The symptoms of poisoning would indicate that the members of this group differ in the mechanism of their action from the DDT group. For example, Busvine (22) contrasts the "fanning" movements of wings of flies poisoned by chlordane or γ -BHC (lindane) with the absence of abnormal wing movement in DDT-poisoned flies, and he quotes Barnes who observed that the widespread muscular twitchings in rats poisoned with DDT is not seen in rats poisoned

by lindane, aldrin, dieldrin, endrin and chlordane. Moreover, Busvine's results indicate that the resistance of houseflies to the latter group of insecticides is quite distinct from DDT-tolerance.

To the chemist, lindane, the gamma-isomer of hexachlorocyclohexane, appears to be in strange company in the hexachlorocyclopentadiene group for the common feature of the latter group is the hexachlorocyclopentene ring. Busvine (22) observed that the end view of the molecular model of these compounds presents a pentagon formed of five chlorine atoms and that, of the isomers of hexachlorocyclohexane, only the gamma isomer reveals a similar pentagonal arrangement. Busvine suggests that this chlorine pentagon may form the toxophore of this group of compounds, but it is tempting to argue that it may be the area of attachment to the biological interface. But the latter hypothesis leaves unexplained the similarity in toxic action of the widely different structures which thus stand out from the interface. Nor does either hypothesis provide an explanation of the poor insecticidal activity either of chlordane or of enneachlor (4,7-methano-3a,4,7,7a-tetrahydro-1,2,3,4,5,6,7,8,8-enneachloroindan).

THE PYRETHRIN GROUP

If, for a few paragraphs, the matter of stereoisomerization may be ignored, the structure of this group of insecticides may be represented in XI. Hydrolysis of these esters yields acidic and alcoholic components; the former are chrysanthemic acid ($R' = -CH_3$) and, following the nomenclature of Harper (23), pyrethic acid ($R' = -CO \cdot OCH_3$). The alcoholic components, R'' substituted cyclopentenolones, are named pyrethrolone, cinerolone, allethrolone, furethrolone, and cycloethrolone.



$R' = -CH_3;$	$R'' = -CH_2 \cdot CH : CH : CH : CH_2$	Pyrethrin I
$R' = -CO \cdot OCH_3;$	$R'' = -CH_2 \cdot CH : CH : CH : CH_2$	Pyrethrin II
$R' = -CH_3;$	$R'' = -CH_2 \cdot CH : CH : CH_3$	Cinerin I
$R' = -CO \cdot OCH_3;$	$R'' = -CH_2 \cdot CH : CH : CH_3$	Cinerin II
$R' = -CH_3;$	$R'' = -CH_2 \cdot CH : CH_2$	Allethrin
$R' = -CH_3;$	$R'' = -CH_2 \cdot C : CH : CH : CH$ 	Furethrin
$R' = -CH_3;$	$R'' = -CH$ 	Cycloethrin

Pyrethrins I and II and cinerins I and II are present in a mixture, amounting to 0.7 to 3.0 per cent in the flowerheads of *Chrysanthemum cinerariaefolium*. Allethrin is an analogue prepared by a synthetic process described by Sanders & Taff (24) involving 12 to 20 separate steps and the handling of the highly explosive ethyl diazoacetate. Commercial production, which began in 1950 with about 8500 lb., reached 50,000 lb. in 1953 at a market price of around \$32.00 per lb. Furethrin, a synthetic product, is described by Matsui *et al.* (25) who used as starting material furfuryl acetone, cheaper to produce than the allyl acetone used in allethrin manufacture.

Chrysanthemic acid can exist in *cis* and *trans* forms, for the hydrogen atoms at carbons 2 and 3 may lie on the same (*cis*) or opposite (*trans*) sides of the cyclopropane ring. Since neither of these positional isomers has a plane of symmetry, each has optical isomers. Crombie & Harper (26) have shown that the naturally-occurring form present in pyrethrin I and cinerin I is the (+)-*trans* isomer. The synthetic acid is produced by the addition of ethyl diazoacetate to tetramethylbutadiene, and the samples reported by Elliott (27) were estimated from infrared spectra to contain about 70 per cent of the *trans* and 30 per cent of the *cis* isomers; these isomers will be present as the racemic mixtures of the optical isomers.

The substituted cyclopentenone ring of the alcoholic component, having no plane of symmetry will exist as optical isomers. Moreover, when the unsaturated side chain R'' bears two different groups on its ethylenic carbon, *cis* and *trans* isomerization is possible. The latter complication does not arise with the synthetic products. Crombie, Harper & Newman (28) deduced that the keto-alcohol present in the natural pyrethrins is (+)-*cis*-pyrethrolone, a conclusion which conforms to the earlier indications [see LaForge & Green (29) that the natural cinerins were esters of *cis*-cinerolone.

A characteristic feature of the action of the pyrethrin group of insecticides is the rapidity of "knockdown" and the comparatively long interval before death. The speed with which the initial reaction occurs reveals rapid penetration and spread along the nerve interface, implying a marked surface activity. A second feature is the high degree of flexibility of the pyrethrin-like molecule, for free rotation is possible not only at the ester linkage but at the bonds between carbons 1 and 2, 3 and 4. This marked contrast to the more rigid molecule of rotenone, nicotine, or aldrin will allow the pyrethrin molecule to fit itself to the biological interface permitting short-range forces full play. If so, it would be expected that the three-dimensional geography of the entire molecule and, particularly, the spacial distribution of the reactive groups will determine degree of toxicity. It is, however, remarkable that the saturation of the double bonds present in the pyrethrin molecule markedly reduces the insecticidal activity as found in the early work of Staudinger & Ruzicka (30); the greater flexibility of the hydrogenated molecule should permit a readier fit with not too great an alteration of molecular dimensions.

It is convenient, in discussing the influence of molecular change on insecticidal activity to begin with the left-hand side of formula XI. At

carbon 5, the replacement of one of the methyl groups by the carbomethoxy group, whereby pyrethrin I and cinerin I become pyrethrin II and cinerin II respectively, reduces potency to about one-quarter, though good "knockdown" properties are retained [Gersdorff (31)]. Saturation of the double bond between carbon 4 and 5 in pyrethrin I and cinerin I reduces toxicity to about half, knockdown being but little affected; Gersdorff (31) found that a similar change rendered pyrethrin II and cinerin II virtually nontoxic. Elliott quoted unpublished results which show that the toxicity of allethrin was reduced by a factor of two or three when this bond was saturated.


The next feature to be considered is the *cis-trans* isomerization at the cyclopentane ring. Staudinger & Ruzicka (32) considered that the pyrethrin containing the (+)-*cis* acid were less toxic than that of the (+)-*trans* acid; Elliott also found that in the corresponding allethrins the esters from the (+)-*trans* acid were almost twice as toxic as those of the (+)-*cis* acid. Optical isomerization exerts a more profound effect on toxicity, for Gersdorff (33) found that in both cinerin I and allethrin the esters of the natural (+) form were about 3 to 8 times as toxic to houseflies as the esters of the racemic mixture. Elliott reported that allethrin prepared from the (-) form was about 50 times less potent than the ester of the (+) form. The same author also said that the replacement of the cyclopropane ring by a double bond between carbon 2 and 3 gave compounds with no measurable toxicity. It would appear that the *gem*-dimethyl group is not an important factor for Staudinger & Ruzicka showed that the esters in which these methyl groups were replaced by hydrogen were insecticidal.

Consider now the features of the alcoholic component in relation to insecticidal activity. The rupture of the allethrolone ring to form 3-hydroxy-8-nonene-2,5-dione, $\text{CH}_3 \cdot \text{CO} \cdot \text{CH}(\text{OH}) - \text{CH}_2 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH} : \text{CH}_2$, markedly reduces toxicity in the ester which retains high "knockdown" activity. The effects of isomerization caused by the asymmetry of the cyclopentenolone ring are not clear cut. LaForge & Green (29) found that, when esterified with (+)-*trans*-chrysanthemic acid, (-)-*cis*-cinerolone gave an ester twice as toxic to houseflies as the ester of (+)-*cis*-cinerolone; Elliott also reported a fourfold increase in toxicity when (+)-*cis*-cinerolone replaced (-)-*cis*-cinerolone. Likewise, he found that the ester of (+)-allethrolone with (+)-*trans*-chrysanthemic acid was about twice as toxic as the corresponding ester of (+)-allethrolone. On the other hand, Gersdorff & Mitlin (34) deduced that when esterified with (+)-*trans* acid, (-)-cinerolone gave esters about five times as toxic as that of the (+) isomer.

It is evident from the number of compounds listed in XI that the choice of the side chain R'' is not critical. Staudinger & Ruzicka (30) discovered that at least one double bond is required in this group to give high toxicity; Gersdorff (31) found that tetrahydropyrethrin I, in which R'' is the *n*-amyl group ($-\text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_3$), was about one-fiftieth as active to houseflies as pyrethrin I, a conclusion confirmed by Crombie, Elliott & Harper (35). The series of allethrin analogues examined by Elliott (27) reveals high

knockdown and toxic properties, greatest when the double bond is between the second and third carbon atom of the side chain. From the latitude thus permitted in the choice of the side chain R'' it is evident that *cis-trans* isomerization when it exists in R'' is not of primary importance in determining insecticidal activity.

The outstanding points emerging from this survey of the relationship of toxicity and structure in the pyrethrin type molecule are, firstly, the greater leniency possible to secure "knockdown" activity. This reversible state is apparently achieved by a loose combination of toxicant and biological interface, a combination which is susceptible to detoxification mechanisms. Herein may rest the significance, at least in part, of the marked effect of the pyrethrum synergists. The second prominent feature is the importance of stereo isomers in the acid component; esters of the $(-)$ acid are much less insecticidal than those of the naturally-occurring $(+)$ acid. A differential toxicity in optical isomers is not uncommon; an early example, from insect toxicology, is nicotine [Richardson, Craig & Hansberry (36)], of which the *l*-form is twice as toxic as the *dl*-form. A recent example, attributable to Hoskin & Trick (36a), is the chemical warfare agent tabun (ethyl *N,N*-dimethylphosphoramidocyanidate) of which the *l*-form is apparently nontoxic. The simplest explanation of this difference is the hypothesis that in such instances an irreversible inhibition of some vital function is possible only when the toxicant becomes coupled at three points, A, B, and C, arranged on the

biological interface, for example, as in the triangle . The toxicant

bears three complimentary reactive groups so arranged that they may be represented by the thumb and the first two fingers of each hand. Now the thumb (A), forefinger (B), and middle finger (C) of the right hand may be placed on A, B, and C above easily, but with the left hand the juxtaposition is impossible except from below the plane of the paper. Similarly, the coupling to the interface of the three reactive groups of the toxicant is possible only with one stereo isomer; the other can become coupled at two points only. Further let it be supposed that the number of sites, A, B, and C on the biological interface is limited and that most must, for lethal consequences, be occupied by the toxic isomer. Under these conditions, if the racemic compound is used, the enantiomorph will occupy half the available sites; it will function as a competitive antagonist. In other words, the potency of the racemic mixture will be half that of the active isomer if the enantiomorph is inactive but will be less than half if the enantiomorph functions as a competitor for the available sensitive sites.

The practical significance of these conclusions is that the chemist in attempting to produce synthetic pyrethrins is at a disadvantage. He may, as with allethrin and furethrin, use an R'' side chain imparting greater in-

secticidal activity than is present in the natural products, but he is always faced with the possibility that, by having to use the racemic acidic component, half of his product will not only be an inferior insecticide but may, under limited conditions, reduce the toxicity of the active half. Fortunately, the evidence to date does not indicate any drastic intervention by the esters of the (-)-chrysanthemic acid.

ORGANOPHOSPHATES

The development of the organophosphates has been profoundly influenced by two factors. The first is that the search for insecticides among these compounds, led by Schrader (37), has been guided by the generalizations he formulated around 1945 to 1946. The second is that the extreme physiological activity of certain of these compounds was, in 1940 to 1941 early in their history, traced by Adrian, Feldberg & Kilby (38) to their inhibitory action on the cholinesterases.

Schrader's observation [see Martin & Shaw (39)] that mammalian toxicity is reduced without marked loss of insecticidal properties by the replacement of the C—P linkage by the C—O—P linkage focused attention on the esters which still form the preponderant group of the insecticidal organophosphates. Schrader considered that the active esters were of structure $(RO)_2PO \cdot Y$ where Y is a group such that the HY has acidic properties. This generalization was given meaning when Jansen *et al.* (40) showed that the inhibition of chymotrypsin by diisopropyl phosphorofluoridate (DFP) was a result of the phosphorylation of the enzyme, hydrogen fluoride being released at the rate of approximately one mole per mole of α -chymotrypsin. The hypothesis that the inhibition of the cholinesterases is similarly attributable to phosphorylation, advanced by Burgen (41), received experimental support from the results obtained by Bournsnel & Webb (42) working with horse serum esterases and radioactive DFP.

If enzyme inhibition is attributable to phosphorylation, the inhibitory power of the ester should be related to its capacity to phosphorylate water, that is, to say, to its rate of hydrolysis. Aldridge & Davison (43) found a linear relationship, in a series of diethyl phenyl phosphates substituted in the aromatic ring, between the logarithm of the bimolecular rate constant of the reaction of cholinesterase with the phosphate and the negative logarithm of the hydrolysis rate constant of the inhibitor. Now if Y is a potentially acidic radicle, the PY bond would be less stable than the alkoxy bond (ROP), and its rupture by hydrolysis involves an energy of the order of 12 to 16 kcal./mole, bringing it within the "energy-rich" category as defined by Lipmann (44). Ketelaar (45) suggested that as the speed of hydrolysis of the esters will be governed by the change of free energy the latter will be proportional to the acid component P_K of the hydrolysis product. He therefore concluded that if HY is an acid weaker than P_K of above 9.0, the diethyl phosphoric ester will be almost inactive in the inhibition of cholinesterase, whereas those of acids of P_K less than 7 will be powerful inhibitors.

The relationship between inhibitory power and ease of hydrolysis must clearly break down if the PY bond is so reactive that water or groupings other than those of the enzyme are phosphorylated. Thus as Burgen pointed out, diisopropyl phosphorochloridate is a more active phosphorylating agent than DFP yet is far less active as a cholinesterase inhibitor. If the ester is stable enough and has the solubility relationships and surface activity necessary for it to reach the enzyme sites, it follows from the phosphorylation hypothesis that the nature and properties of the inhibited enzyme are determined solely by the alkoxy (RO) groups irrespective of the Y groups present in the inhibitor, a corollary found true by Aldridge & Davison (46). By the chromatography of the hydrolysate of chymotrypsin or cholinesterase inactivated by DFP, Schaffer, May & Summerson (47) isolated L-serine phosphoric acid, indicating that phosphorylation occurs at the hydroxyl group of a serine residue. Because there are about 27 serine residues in the chymotrypsin molecule and, as stated above, the reaction is mole per mole, and because many other proteins regardless of their serine content are not phosphorylated by DFP, it would appear that but one serine group of the enzyme is able to react, presumably because of the particular configuration of the amino acids in its vicinity. In contradistinction to DFP, diisopropyl phosphorochloridate readily phosphorylates the amino group of serine or of imidazole. The hydroxyl group of the serine made reactive by its surrounding configuration therefore represents the active group whose phosphorylation destroys the ability of the enzyme to accelerate the hydrolysis of choline esters; consequently the substrate-enzyme complex must either involve this particular serine or the phosphorylation of the latter must prevent, for example, by steric hindrance, the approach of the choline esters to an adjacent active center of the enzyme.

If the phosphorylation of the cholinesterases were the only factor determining toxicity, the prospect of discovering organophosphates of low toxicity to mammals would be slight. Reliance would have to be placed almost entirely on solubility factors using, for example, the more effective passage of lipophilic compounds through the insect cuticle than through the human skin. Ketelaar (45) suggested that the general observation that thiophosphates are relatively more toxic to insects and less toxic to mammals than the organophosphates is a reflection of the high polarity of the $P=O$ bond which induces in phosphates a greater water solubility and poorer lipid solubility than in the corresponding thiophosphates.

The thionophosphates differ from the corresponding phosphates in properties other than lipid solubility; parathion (diethyl *p*-nitrophenyl thiophosphate) was shown by Diggle & Gage (48) to cause no measurable inhibition of rat brain cholinesterase *in vitro*. Similarly, Hodge and his colleagues (49) found freshly purified EPN (ethyl *p*-nitrophenol thionobenzene phosphonate) without *in vitro* anticholinesterase activity.

The replacement of the phosphoryl group ($P=O$) by the thiophosphoryl group ($P=S$) is known to reduce markedly the rate of hydrolysis of the

esters, yet the thionophosphates are converted in the animal to an active anticholinesterase. It might at first seem probable that this conversion is associated with the tendency of the thionophosphates to change spontaneously or by heat treatment to the thiol isomer. In 1922 Emmett & Jones (50) reported that 0,0,0-trimethyl thionophosphate was converted, on heating at 100°C. in a sealed tube, to the 0,0,S-isomer. Bennett *et al.* (51) reported the loss of thionosulphur and of insecticidal properties in heat-treated parathion. Gardner & Heath (52) showed that the systemic insecticide "Systox" is a mixture of demeton (0,0-diethyl, 0-ethylmercaptoethyl thionophosphate) and its S-ethylmercaptoethyl isomer. Consideration of the biological activity of the thionophosphates therefore involves consideration of the thiol isomers. Aldridge & Davison (43) found that parathion and its S-phenyl isomer follow the rule that the stability to hydrolysis is inversely related to *in vitro* inhibitory properties; their results with the S-ethyl isomer are suspect on the score of purity of the samples used. The S-ethyl isomer was found by Metcalf & March (53) to be rapidly hydrolysed by normal sodium hydroxide and correspondingly more inhibitory to the brain cholinesterases of both the fly and the mouse. The comparative toxicology of the isomers reveals startling differences. The approximate lethal dose by Aldridge & Barnes (54) was as follows: parathion 3 mg./kg.; S-ethyl isomer 1.2 mg./kg.; S-phenyl isomer 0.5 mg./kg., compared to that of para-oxon 0.4 mg./kg. The same order of toxicity was found by Callaway, Dirnhuber & Wilson (55) for the shrimp *Gammarus pulex*. As insecticides, Stringer [quoted by Martin (56)] found that to *Diataraxia oleracea*, the S-phenyl isomer was less toxic than the S-ethyl isomer which, in turn, was less toxic than parathion. Potter (57) reported that against four insect species the order of toxicity was parathion, S-ethyl isomer, S-phenyl isomer; this is the reverse of the order of toxicity to rats as reported above. The intervention of the biological process by which the thiophosphates are converted to active anticholinesterases has created a differential toxicity to insects and to mammals, thus pointing a possible route to organophosphates of low poison hazards.

The conversion of parathion to an active compound by liver slices was shown by Gage (58) to involve not isomerization but oxidation. Myers *et al.* (59) advanced evidence that the active oxidation product in rat brain was para-oxon; Kok & Walop (60) found that the fat body of insects was the tissue most effective in this conversion, para-oxon again being favored as the active product.

In vivo oxidation appears also to be involved in the conversion to an effective insecticide of schradan (octamethylpyrophosphoramidate) which is a poor inhibitor of cholinesterase *in vitro*. Following up the suggestion of Hartley (61) that the active anti-enzyme is produced by the oxidative addition of the nitrogen atom of schradan, Casida, Stahmann and their colleagues (see 62) and O'Brien & Spencer (63) have shown that the effective product formed in insects, mammals and, to a slower extent, in plants, is the phos-

phoramide oxide. The oxide is readily hydrolysed and thus follows the rule relating inhibitory power to ease of hydrolysis. If, in the animal body, the amide oxide is formed at an insensitive site, hydrolysis may intervene before the opportunity of phosphorylation of the cholinesterases. This possibility is used by O'Brien & Spencer (64) to explain the selective insecticidal action of schradan which is nontoxic to leaf-eating insects. This example is quoted to illustrate a still further possible route to selective insecticides and to organophosphate insecticides of low mammalian toxicity. Schradan has, however, been investigated primarily because of its activity as a systemic insecticide, a subject which will not be followed here because it has been recently fully reviewed by Tew & Kirby (65) and by Spindler (66).

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PERSISTING INSECTICIDE RESIDUES IN PLANT MATERIALS¹

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Nonionic organic insecticides² are soluble in plant oils and waxes and therefore may be expected to penetrate in part at least into cuticular and often into subcuticular tissues of treated plant parts (see Table I). Since penetrated insecticides are not easily removed from within these tissues,

TABLE I
EXAMPLES OF PENETRATION OF INSECTICIDES AND ACARICIDES INTO
TREATED PLANT PARTS

Plant Part	Material	Formulation	References
Apple foliage	DDT	Solutions	1, 2
Apple fruits	DDT	Emulsions	18, 25, 72
Avocado foliage	DDT	Solution	10
Avocado fruits	DDT, dieldrin	Emulsions	3
Blackcurrant fruits	DDT	Emulsions	2
Cabbage foliage	Parathion	Many	19
Cherry fruits	Parathion	Emulsions	15
Citrus foliage	DDT, parathion	Solutions	4, 13
Citrus peel	Aramite, Chlorobenzilate, demeton, dieldrin, EPN, malathion, Ovotran, parathion, schradan	Many	5 (p. 139), 6 to 9, 13
Coffee plants	DDT	Solution	10
Olive fruits	Parathion	Many	11, 12
Peach fruits	DDT	Emulsions	18, 25, 72
Pear fruits	DDT, parathion	Many	14, 18, 25, 72
Potato foliage	DDT	Emulsions	2
Potato tubers	BHC, lindane, parathion	Suspensions	15
Strawberry fruits	Parathion	Emulsions	15
Unspecified foliage	BHC, DDT	Many	1, 16, 17

¹ The survey of the literature pertaining to this review was completed in June, 1955.

² In this review common names of insecticides and acaricides are used in accordance with the directive of the Committee on Insecticide Terminology of the Entomological Society of America [*J. Econ. Entomol.*, **48**, 112-15 (1955)]; the reader is referred to this directive for definitions of usage. Also, in this review both insecticides and acaricides will usually be designated simply as insecticides.

we may expect repeated exposures to insecticide residues by ourselves and by our livestock. This has been demonstrated by Walker *et al.* (20), who detected DDT and DDT-ethylene in small amounts in 25 restaurant and institutional meals. The best appraisal of any hazard that may exist from the consumption of treated crops would include the accrual of degradation and persistence residue curves and both qualitative and quantitative information about ultimate *in situ* residue metabolites as well as appropriate toxicological interpretations of the residue data. Obviously, adequate analytical methods are required.

The present review is concerned with the evaluation of the quantitative significance of this situation with regard to the magnitudes, locales, and *in situ* fates of residues of insecticides and acaricides on and in field-treated foodstuffs and feeds of plant origin. Toxicological interpretations of the data are not included.

A general discussion of some analytical problems related to the presence of insecticide residues on fruits and vegetables has been prepared by Porter & Fahey (21), and Boswell (22) has reviewed the effects of soil residues upon subsequent generations of crops grown therein. Gunther & Blinn (5) have recently discussed in detail many analytical aspects of the present concern about persisting residues of insecticides and acaricides. Dormal (15) has very thoroughly summarized to date nearly all published insecticide residue data from the United States and from France on all crops.

CHARACTERISTICS OF RESIDUES

Insecticide deposits and insecticide residues are not synonymous. Gunther & Blinn (5) use the word "deposit" in reference to the insecticide as initially laid down on a surface by the treatment, and the word "residue" in reference to the insecticide regardless of locale on or within a substrate and with the implication of aging (time lapse or alteration or both). Thus, a deposit becomes a residue as soon as affected by weathering, metabolic degradation, and other processes.

The magnitudes of initial deposits are influenced by many factors, including dosage, nature of substrate, formulation characteristics [see Ebeling (23); Hardman & Thomas (24)] and uniformity of application [see Gunther & Blinn (5)]. As soon as deposited material not adhering tenaciously to the plant surface sloughs off, the initial deposit becomes an effective residue, then a residue. In turn, residues have been classified by Gunther, Barnes & Carman (25) as extrasurface, cuticular, and subcuticular, depending upon whether the insecticide is still adhering to the waxy cuticle, is embedded or dissolved in it, or has penetrated into the plant part below the cuticular layers.

Residues of insecticides tend to disappear or otherwise lose their analytical identity at a constant rate which is a function of concentration; the percentage or fractional decreases, however, are independent of initial concentration or magnitude of deposit [Gunther & Blinn (5); Decker (26);

Satyanarayana (1)). The initiation of this type of behavior is degradation (degrading residue) which demonstrates weathering as contrasted with subsequent losses which are the result of enzymatic or other alteration of the parent insecticidal molecule inside the plant part (persisting residue). For most substrates the two types of behavior are indistinguishable and obviously merge; for citrus peel both types are demonstrable [Gunther & Blinn (5)]. It is doubtful if simple sublimation or any other simple mechanism is the only explanation for decreasing residues and decreasing residual effectiveness on plant surfaces, as implied by Wasicky & Unti (27).

Three conclusions may be drawn from these empirical characteristics of residues. First, penetrated residues are not removable by washing the crop [Gunther *et al.* (25)], although some deposits and extrasurface residues are removable [Gunther *et al.* (25); Smith *et al.* (28, 55)] or at least reducible in amount. Second, degrading and persisting residues appear to follow first-order reaction kinetics and may therefore be plotted as straight lines of log deposit, log parts per million, or fraction of loss or retention directly against time elapsed since treatment. As in all first-order reactions, the period of half-life, or the time required for any given fraction to react or dissipate, is independent of the initial concentration and therefore represents a characteristic of each insecticide on or in a particular substrate. This concept in its present application has been discussed by Gunther & Blinn (5); the utility of this application promises to be considerable in predicated longevity of residues, in precisely evaluating effects of additives, adjuvants, and amendments, and in establishing magnitudes of optimum initial deposits [see Gunther & Blinn (5)]. Third, there can be no doubt that some, if not most, of the penetrated residue is metabolized or otherwise altered chemically within the plant part; the importance of the establishment of the nature and the fate of such penetrated and aged residues is discussed in a later section of this review.

MAGNITUDES OF RESIDUES

Tolerances.—At the present time the unit magnitudes of residues, either extrasurface or penetrated, are important and necessary in the establishment of tolerances for the ultimate residues [Gunther *et al.* (25)] of new insecticidal materials. If residue tolerances for established insecticides are exceeded, the raw agricultural commodity involved will be seized. These developments originated with the 1950 Hearings (Section 406 of the Food, Drug, and Cosmetic Act) followed by the Miller bill which was signed by the President on July 22, 1954 and which becomes Section 408 of the Food, Drug, and Cosmetic Act, effective July 22, 1955 [Palm (29)]. Presently effective tolerances and exemptions based upon the Hearings in 1950 are listed in detail in the *Federal Register* for March 11, 1955, pp. 1473–1508, by the Food and Drug Administration, Department of Health, Education, and Welfare. Under the Miller Amendment tolerances are set for each raw agricultural commodity as it is found because "it is impractical to establish tolerances on

the basis of the dry matter" of fruits and vegetables. However, the proposal of Baier & Wilson (30) for establishing tolerances has much merit and will undoubtedly receive further evaluation. Their proposal considers the readily determinable nonaqueous matter as the most practical index of food consumption, and it

provides for a safe upper limit or tolerance based on the nonaqueous matter while retaining a nominal tolerance quite similar to present practice. It will be readily seen that the proposed basis is equally adaptable to the whole commodity as purchased or to the edible portion, whichever may be determined by future consideration.

This procedure would put broad classifications of foods under regulations that are worded the same and mean fundamentally the same thing from the points of view of producer, processor, consumer, and law enforcement agency.

Dormal (15) has very completely discussed the insecticide and other pesticide residue tolerance and regulatory situations of 1954 status in Great Britain, France, West Germany, Austria, Holland, Switzerland, and Greece, as summarized in Table II. She also discusses regulatory measures in the United States prior to the recent announcements cited above.

TABLE II
INSECTICIDE RESIDUE TOLERANCES IN OTHER COUNTRIES

Country	Materials	Tolerance, p.p.m.	Status
Great Britain	DDT	7	Recommended
	Dimefox (Hanane)	<0.2	Proposed
	Isopestox†	<1	Proposed
	Lindane	2.5	Recommended
	Schradan	<3	Proposed
France	Arsenic	2.7*	By law
	DDT, BHC, TEPP, parathion, others	None*	By law
	Arsenic	None*	By law
West Germany	DDT, demeton, parathion	None*	Recommended
Austria	Lead, arsenic, thiophosphoric esters	None*	By law
Holland	Parathion, schradan, Systox, TEPP, several fumigants	None*	By law
Switzerland	Aldrin, chlordane, DDT, Diazinon, lindane, parathion and related esters, systemics	None*	Recommended
Greece	Parathion	None†	By law

* Timing of application on specific crops recommended or regulated, or certain materials on certain crops prohibited.

† Use of parathion on olives regulated.

‡ Bis(isopropylamino)fluorophosphine oxide.

Typical residue data.—In Table III are collated typical residue data from the available literature. Only the organic insecticides and acaricides are included.

TABLE III

EXAMPLES OF MAGNITUDES, DEGRADATION HALF-LIVES, AND TOLERANCES OF INSECTICIDES AND ACARICIDES ON AND IN TREATED PLANT PARTS

Material	Plant Part	Magnitude, p.p.m.		Half- Life, Days	Toler- ance p.p.m.*	Refer- ence
		Initial	14 Days			
Aramite	Citrus foliage	3.3†	1.2†	9	—	31
	Citrus peel	0.35§	0.18§	7	—	32
	Cotton foliage	2.0	Trace	1	—	31
	Lima bean foliage	1.4	Trace	1	—	31
BHC	Alfalfa	17	4.6	7	—	33
Chordane	Alfalfa	12	4	9	—	34
Chlorobenzilate	Citrus peel	11	8.5	66‡	—	35
DDT	Alfalfa	13	5	6	—	33
	Citrus foliage	35	27	39	—	5 (p. 6)
	Citrus peel	40	25	26	7	5 (p. 6)
	Clover	280	125	12	—	36
	Lettuce	80	1.3	2	7	37
	Peach fruits	8.6	6	28	7	38
	Peach foliage	290	125	12	—	36
	Citrus peel	0.30	0.22	30	—	39
	Pea plants	26	3.5	5	—	40
	Potato tubers	0.25	0.13	16	—	39
Dieldrin	Apple foliage	110	41	10	—	36
	Citrus fruit	1.7§	0.4§	2,60‡	—	41
	Clover	40	9	6	—	36
	Peach fruits	6.5	1.9	8	0.1	42
	Peach foliage	25†	6.5†	7	—	42
EPN	Apple foliage	75†	3†	3	—	43
	Peach fruits	9.5	3.4	10	3	43
	Peach foliage	10.5†	2.1†	6	—	38
Ethylene chloro- bromide	Citrus peel	4.0	1.5	3,100‡	—	44
Ethylene dibromide	Citrus peel	11.0	1.5	1,7‡	—	44
Heptachlor	Citrus peel	4.5	1.0	4,23‡	—	45
Lindane	Alfalfa	7.4	0.7	4	—	33
	Clover	31	3.6	3	—	46
	Apple fruits	4	0.3	3	—	47
Malathion	Apple foliage	2.8†	Trace	3	—	47
	Citrus peel	4.0	1.0	5,17‡	—	13
	Date fruits	8.2	1.4	6	—	48
	Lettuce	40	Trace	2	—	49

TABLE III—continued

Material	Plant Part	Magnitude, p.p.m.		Half- Life, Days	Toler- ance p.p.m.*	Refer- ence
		Initial	14 Days			
Methyl bromide	Onion bulbs	11	Trace	2	—	49
	Peach fruits	9	1.2	5	—	42
	Tomato fruits	10	Trace	2	—	49
	Avocado fruits	11.3	6.8	1,44†	—	50
	Citrus peel	8.5	4.1	1,56†	—	50
Methyl parathion	Apple foliage	40	2	3	—	43
Ovotran	Citrus peel	6.4	3.0	5,30‡	—	8
Parathion	Alfalfa	1.5	0.2	6	—	33
	Apple fruits	4	0.8	6	1	51
	Apple foliage	60†	2.0†	2	—	43
	Celery	5.5	0.17	3	1	52
	Citrus peel	16	3.8	7	1	72
	Date fruits	8.2	1.4	6	1	53
	Lettuce	2.2	Trace	2	1	37
	Peach fruits	3.5	0.9	7	1	43
	Peach foliage	0.6†	0.02†	2	—	38
	Strawberry fruits	0.6	Trace	3	1	15
	Strawberry foliage	10	0.65	4	—	15
	Tomato fruits	0.95	0.15	7	1	49
Schradan	Pea plants	210	11.5	4	—	54
Sulfotepp	Lettuce	25	Trace	1	—	55
	Tomato foliage	10	Trace	2	—	55
Toxaphene	Alfalfa	30	16	16	—	34

* From the *Federal Register*, 20, 1502-8 (1955).† $\mu\text{g./cm.}^2$

‡ Persisting residue.

§ Based upon weight of whole fruit.

Included in this table are data for initial deposits as well as for the persisting residues 14 days after application. Initial deposit values afford an indication of the dosage used, while the residue value at 14 days affords a realistic evaluation of the persistence behavior of the material on a given substrate. In most instances the available data did not include specifically the 14-day interval; this was derived by constructing the persistence curve of residue versus elapsed time. Half-life values were similarly derived from the published data [see Gunther & Blinn (5)]. It should be noted that the unstarred half-life values are degradation half-lives.

From the data in this table, the residue remaining on the plant part at any time after application may be calculated with a reasonable degree of assurance. Where available, tolerance values have also been included for comparison purposes.

Analytical problems encountered in obtaining reliable residue data are recounted in a later section of this review.

LOCALES OF RESIDUES

As physiologically active substrates, plant parts may absorb and translocate topically applied insecticidal chemicals. Cassida & Allen (56) and Crafts (57) have reviewed the mechanism of plant absorption and the structure of plant cuticle in this connection.

Nonpenetrated residues.—Residues of most inorganic and certain ionic organic insecticides do not penetrate appreciably into the waxy or oily cuticular tissues of plant parts such as leaves and fruits. It is conceivable that these materials might penetrate into structures such as aged bark and root tissues, but there seems to be no specific information available on this point other than possibly with some of the selenium-containing compounds [Neiswander & Morris (58)], with some of the arsenic-containing formulations [Cassida & Allen (56)], and with sodium fluoroacetate [Cassida & Allen (56)].

As pointed out earlier, deposits of organic insecticides seem to penetrate in part and very quickly into cuticular and subcuticular regions of leaves and fruits. Sometimes initial deposits may be removed by washing [Gunter *et al.* (25); Smith *et al.* (28, 55)] although the presence of oily emulsifiers and carriers should prevent ready removal even of initial deposits [see Ebeling (23); Fahey *et al.* (59); Ginsburg *et al.* (46)].

Penetrated residues.—Most insecticide residues that will be encountered are penetrated residues, including those initially laid down from aerial application, from injection, brushing, or painting, and from absorption from soil treatments (see Table IV). There can be no question that penetrated residues, if still available to the insect pest of interest, afford desirable [Cassida & Allen (56)] and perhaps even prolonged insect protection.

There is some evidence that leafy tissues will absorb insecticides subcuticularly, as previously shown in Table I. Whether there is a distinction in penetration behavior among leaves with xeromorphic structures (citrus), mesomorphic structures (apple, peach), and succulent structures (cabbage, celery, lettuce, spinach) is not clear. From the data underlying Table I it would seem that the major categories of leaf structure do not differ much in absorption of insecticides; there are almost no data for succulent structures. The accrual of penetration data for leaf tissues is analytically very difficult because of stripping solvent-induced penetration during the processing operations. In this connection, the "used-droplet" residue technique of Fransen & Kerksen (63) may prove to be generally useful; they ran droplets of sprays or suspensions over cabbage leaves and analyzed the run-off material versus starting material to establish the very fast penetration of parathion into the cabbage leaf waxes.

More reliable analytical results are possible with most fruits and non-leafy vegetables, and penetration residue data with a large variety of these substrates will be acquired rapidly during the next few years.

TABLE IV
EXAMPLES OF ABSORPTION OF SOIL INSECTICIDES BY ROOT CROPS,
ONE YEAR AFTER TREATMENT

Material	Plant Part	Dosage†	Residue, p.p.m.	Reference
Aldrin	Carrots	4.7	0.4	60
	Potatoes	10	0.05	61
BHC	Potatoes	10	0.15	61
Chlordane	Carrots	9.4	1.4	60
	Potatoes	10	0.08	61
DDT	Carrots	19.5	0.5	60
	Potatoes	10	0.02	61
Dieldrin	Carrots	6.0	0.7	60
	Potatoes	10	0.03	61
Endrin	Carrots	4.9	0.7	60
EPN	Potatoes	10	0.01	61
Heptachlor	Carrots	5.0	0.9	60
	Potatoes	10	0.18	61
Lindane	Carrots	0.9	1.0	60
	Peanuts*	1-5†	0.1	62
Parathion	Cucumbers	8-64	0.02	55
	Potatoes	10	<0.01	61
	Tomatoes	8-128	0.01	55
Toxaphene	Carrots	19.6	1.0	60
	Potatoes	10	<0.04	61

* Peanut butter.

† Peanuts grown after cotton foliage was treated with this dosage.

‡ Pounds of actual compound or active mixture per six inch acre of soil.

Penetration or absorption into plant parts grown in insecticide-treated soils is considered in the following section.

Translocated residues.—This category includes residues from topical, injection, and soil applications.

With the exceptions of selenium, arsenic, and fluoroacetate ion mentioned earlier, there is little reliable direct chemical evidence that many of the currently used insecticides migrate or translocate unchanged any distance in a plant. Much effort is being allocated this problem, however. Indirect chemical and biological evidence is accumulating that systemic insecticides translocate from one portion of a treated plant to another, and some soil insecticides are absorbed by certain root crops.

Residue problems resulting from the use of systemic insecticides have recently been reviewed by Reynolds (64); residue data are listed in Table III. Chemical or equivalent proof that some of the other insecticides occasionally suspected of being "systemic" in their action (e.g., parathion) actually translocate more than a millimeter or two into the plant is almost entirely lacking (viz., parathion into cucumbers, Table IV). Perhaps the distinction between "penetration" and "translocation" is not clear.

There has recently been accumulating evidence that some soil insecticides are absorbed by certain root crops, as shown in Table IV. The evidence has not been presented that this situation is greatly different from ordinary topical application, that is, that the insecticide actually contacting the growing root or tuber is merely penetrating into the tissue making contact. It will be of interest systematically to ascertain depth of penetration as well as possible translocation to the aerial portions of the plants involved. Thus, from the analyst's viewpoint, supporting evidence will be required to establish the significance of the minute amounts of parathion reported in the cucumber and tomato fruits listed in Table IV.

Analytical methods more definitive than the so-called "total" methods will be required [Gunther & Blinn (5)]. In this connection, Terriere & Ingalsbe (61) have reviewed the evidence for the retention of insecticides by corn and potatoes from soils treated with BHC or parathion, with both chemical and biological assay measurements.

Terriere & Ingalsbe (61) found that residues of aldrin, BHC, chlordane, DDT, dieldrin, and toxaphene persisted in sandy loam for at least three years but absorption of these insecticides into carrots, squash, string beans, and tomatoes did not occur. Anderson & Gunther (65) have not found any of the chlorine-containing insecticides listed in Table IV in head lettuce grown in treated soils.

Miscellaneous residues.—Indirect product commodities made from insecticide-containing crops may be expected to retain at least part of the original residues. Among these commodities would be included cattle feeds incorporating citrus peel or sugar beet pulp or cottonseed meal, and various plant and seed oils of commerce. Available residue data for some of these products are listed in Table V. It should be borne in mind that these data cannot be considered typical except perhaps for the olive oil and the wheat

TABLE V
EXAMPLES OF INSECTICIDE RESIDUES IN MISCELLANEOUS COMMODITIES

Commodity	Material	Dosage, lbs. actual/acre	Residue, direct p.p.m.	References
Alfalfa hay	Aldrin	0.125	<0.1	34
	Chlordane	1	1	34
	Toxaphene	1.5	5	34
Cattlefeed, citrus peel	DDT	10	ca. 5	66
	Parathion	10	<1	66
Citrus oil (orange)	Parathion	10	ca. 20	66
Cottonseed extractives	Chlorthion	10	<0.01	67
Marmalade (orange)	Parathion	10	ca. 0.1	66
Olive oil	Parathion	—	ca. 7	12, 68
Wheat bread	Ethylene dichloride	—	Trace	69
Wheat flour	Methyl bromide	95 g./140 lbs.	ca. 40	70

products because data are lacking to establish either ranges or averages of residues.

Discussion of residues in animal products is beyond the scope of this review.

FATES OF RESIDUES

Very little attention has been allotted the establishment of either the nature or the fate of aged insecticide residues within a plant part [Gunther & Blinn (5)]. In 1947 Gunther (71) demonstrated the occurrence of DDT-ethylene and other products in aged, penetrated residues of DDT in citrus leaves and suggested that the toxicology of this derivative should be established along with that of the parent DDT itself. Subsequently, Carman *et al.* (72) presented spectrophotometric information to indicate that both DDT and parathion undergo chemical changes *in situ* in the peel of navel oranges. More recently demeton and schradan have been investigated with regard to metabolism in the plant; in both instances oxidation appears to be involved, with demeton affording a sulfoxide or sulfone [Metcalf *et al.* (73)] and with schradan affording a phosphoramidate nitrogen-oxide [Kilby (74); Hartley (75); Cassida *et al.* (76)].

In general, the fates of persisting residues seem to involve simple mechanical losses as well as degradative losses. The former losses would include volatilization, sloughing off, washing by rainfall, and others similar in nature after reissuance to the surface [see Gunther & Blinn (5)]; the latter losses would include both metabolic and photochemical degradation. The only published information sequestering any of these factors is for demeton and schradan (see above); Carman *et al.* (77) have demonstrated that the nearly 80 per cent loss in the total parathion content of field-treated oranges after 20 days at room temperature did not involve volatilization of this insecticide from the fruits.

ANALYSIS OF RESIDUES

This topic has been previously covered by Gunther & Blinn (5), who conclude that the obtaining of reliable residue data is not a simple matter and involves careful consideration of every possible source of error from the design of the experimental plot to the final tabulation of results. To illustrate, they include discussions of presampling and sampling considerations, sample processing, preanalysis or cleanup treatment, the analysis itself, and the interpretation of the residue data.

Other very useful and recent compendia mentioning residue methods include the annual review in *Analytical Chemistry* (78), a publication of the Pesticide Control Officials (79), Martin's guide (80), and the annotated bibliographies of Frear *et al.* (81).

There can be no justifiable doubt that the careful adaptation of instrumental methods to studies of insecticide residues has resulted in many new accurate and reliable approaches in helping evaluate magnitudes, locales,

and natures of residues; analytical problems as complex as these cannot be solved with minimal equipment. Until residue analysts evaluate their equipment and techniques in terms of efficiency and reproducibility with field-treated samples, however, it will be questionable practice to draw fine comparisons among sets of quantitative data from several laboratories [see Gunther & Blinn (5)].

The so-called "total" methods of analysis, as for chlorine, phosphorus, or sulfur, establish the maximum number of molecules (or weight) of parent insecticide that could be present in a treated plant part regardless of alterations from metabolism or degradation. Fragmentation or other alteration of the parent molecule *in situ* will afford falsely high (apparent) residue values, but these values will not be high in direct proportion to the number of fragments present [Gunther & Blinn (5)]. On the other hand, the so-called "specific" methods are rarely specific for more than a single functional group and may mislead positively from normal plant constituents and negatively from the metabolic masking of the functional group of interest. Clearly, residue data by both types of methods in parallel would provide maximum information well worth the added effort. In the event that metabolism or other degradation is indicated, the addition of companion ultimate bioassay measurements of the residues would prove valuable in appraisals of possible toxic hazards to be associated with the altered insecticide.

The so-called "field" methods, such as the one for DDT residues by Amsden & Walbridge (82), would appear to have merit in comparing efficiencies of deposition as well as in studying the sloughing of initial deposits as they are converted into effective residues.

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REPELLENTS¹

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Introduction.—The bulk of research on repellents was stimulated by the military requirements of World War II and has been continued since then principally with these requirements in mind. Few investigations had been undertaken prior to the war. The results of these investigations together with a history of repellents were reviewed by Dethier (22). Work done by British units just prior to and during the early war years was reviewed by Christophers (19). Work done in the United States by and for the Armed Forces during the same period is tabulated chiefly in the papers of Haller (64), King (73, 74, 75), Morton *et al.* (91), Finkelstein & Schmitt (40), Travis *et al.* (127), and Travis & Smith (128). Additional reviews which also contain information on commercially developed repellents are those of Gil (51), Utzinger (131), and Lesser (79). All of the aforementioned papers treat almost exclusively of repellents for use against blood-sucking arthropods. Accounts of the status of mothproofing since 1946 are given by Lindgren (80), Luttringhaus (84), Stoves (120), Bloomfield (9), Borghetty (10), Borghetty *et al.* (11), Utzinger (131), Zinkernagel (144), and Matsui (89). Wood preservatives are discussed in the papers of Schulze (106), Becker (6), Sedziak (107), Wolcott (138, 143), and in an article in the *Caribbean Forester* (2).

Before the wartime period of intensive investigation there were four standard repellents for use against biting arthropods: oil of citronella, dimethyl phthalate, Indalone, and Rutgers 612. Oil of citronella was the most widely used repellent from about 1901 and was the repellent against which new compounds were tested. Dimethyl phthalate (dimethyl benzene orthodicarboxylate) was originally reported as a fly repellent in 1929 (U. S. Patent 1,727,305). Indalone (*n*-butyl mesityl oxide oxalate or α - α -dimethyl- α -carbobutoxy- γ -dihydropyrone) was reported in 1937 (U. S. Patent 2,070,603). Rutgers 612 (2-ethyl hexanediol-1,3) was the eventual development of a long testing and screening program begun in 1935 by Granett [Granett & Haynes (59)]. At the start of the war the last three compounds were combined in a mixture designated as 6-2-2 and used as a standard all-purpose military repellent. The proportions of the components were: six parts of dimethyl phthalate, two parts of Indalone, and two parts of Rutgers 612. It was partly the failure of this formulation to provide the protection demanded by military requirements which was the stimulus to increased and accelerated research on repellents.

The major share in the accelerated program fell to the Orlando Labora-

¹ The survey of the literature pertaining to this review was completed in June, 1955.

ories of the United States States Department of Agriculture. Between 1942 and 1947 from 5000 to 6000 candidate repellents were screened by these laboratories [Haller (64); Morton *et al.* (91)]. To 1952 approximately 11,000 candidate repellents, insecticides, and rodenticides were screened [King (75)]. The total is currently in excess of 20,000. Less than 10 per cent showed repellency equal to or greater than existing standards, and only three per cent of the promising materials were considered by the United States Food and Drug Administration to be safe for use on man [Travis & Smith (128, 130)]. Of 1339 solid repellents tested at one stage, approximately 9 per cent were outstanding [Linduska & Morton (82)]. Two of the outstanding repellents developed by this program were M-2020 [Smith *et al.* (113)] and M-1960 [Smith & Cole (110)]. At the present time the standard all-purpose skin repellent mixture used by the Armed Forces is M-2020, which consists of dimethyl phthalate 40 per cent, Rutgers 612 30 per cent, and dimethyl carbate (bicyclo [2.2.1]-5-heptene-2, 3-dicarboxylic acid, *cis*-, dimethyl ester) 30 per cent. This mixture as applied is effective for about two hours against anopheline mosquitoes and for about four hours against *Aedes*, ticks, and chiggers. The standard clothing repellent, M-1960, consisting of benzyl benzoate 30 per cent, *n*-butylacetanilide 30 per cent, 2-butyl-2-ethyl-1,3 propanediol 30 per cent, and "Tween 80" 10 per cent is effective when applied at the rate of 2 gm./sq. ft. of cloth for about 7 days against *Aedes*, chiggers, ixodid ticks, and fleas. Recent tests by Smith & Gilbert (114) of modifications of the standard M-1960 have shown that mixtures with undecylenic acid are superior against *Aedes* but inferior against *Anopheles quadrimaculatus*. Species differences of this sort are well known [Travis (125)]. Replacement of *n*-butylacetanilide with *n*-propyl- or *i*-propylacetanilide caused no change in effectiveness. None of these formulations are available for civilian use. Nor have the following highly effective tick repellents been cleared for civilian use: *n*-butylacetanilide, *n*-propylacetanilide, undecylenic acid, and hexyl mandelate [McDuffie & Smith (86); cf. also Granett & French (58)]. Indalone, dimethyl phthalate, and Rutgers 612 are still the repellents of choice [cf. also Cole & Smith (20)].

The avowed purpose of most of the aforementioned work was to develop as speedily as possible a nontoxic [cf. Goldman (53)], nonplasticizing, non-irritating repellent of long lasting efficiency which would repel mosquitoes, biting flies, ticks, fleas, and chiggers. Consequently, most of the tests were screening tests run on a field and laboratory scale, and little thought was given initially to refinement of testing methods and standardization. Few serious attempts were made to investigate the mechanism of repellency or the relation between chemical constitution and repellency. Almost nothing was known of the behavioral and physiological aspects of the problem.

Variables encountered in testing repellents.—In designing tests of repellency it is imperative that one first ask himself what information is being sought. If one wishes to determine how well a compound or formulation operates under actual field conditions, obviously a field test is indicated. If, on the other hand, one wishes to screen candidate repellents, compare rela-

tive effectiveness, or relate repellency to chemical properties, a laboratory test is indicated. Clearly, tests should be carried out with full realization that the results can have significance only if all variables are rigidly controlled. Although Travis (124) stated, "A review of the literature shows that the wide ranges in repellent times seem to be characteristic of tests with repellents against biting insects," Pijoan *et al.* (99) had very narrow ranges in their tests. The presence of wide ranges in repellent times shows only that tests are not precise, not that there is an inherent variability which cannot be controlled. There is no *a priori* reason why a greater basic variability should exist in repellent tests than in toxicity tests or any other type of biological experiment. The need for precision, caution, and control of variables was pointed out very early and very forcefully by Christophers (19) but has been largely ignored.

The variables include not only the obvious ones such as temperature, relative humidity, and light intensity, but more intangible ones such as biting rate, nutritional state, amount of activity and disturbance, behavioral peculiarities, age, and state of development (e.g., nymphal ticks are more sensitive than adults [Brennan (12); Smith *et al.* (111)]), sex, brood differences, sample differences, and a whole group of variables associated with differences in host attractiveness.

The importance of biting rate was first described in detail by Granett (55) who showed that the period of protection offered by a repellent decreased as the biting rate increased [cf. also Travis & Smith (128)]. The decrease is not directly proportional since there is a sharp break at 10 bites per min. Over the range studied, from 0 to 25 bites per min., the ratio of repellent times for any two repellents appeared to be approximately constant. It would be of value to extend the observations to the higher biting rates commonly experienced in the arctic.

All of the factors which affect biting rate are by no means well known. Travis *et al.* (126) and many others have observed that insects do not bite uniformly all day. Christophers (19) noted that the fierceness of attack varied with species and "other conditions" (*sic!*) but could detect no difference in settling which could be correlated with season, strain of mosquito, or the identity of the human host. On the other hand, Terzian & Stahler (122) found that the biting rate was related to the population density at which *Anopheles quadrimaculatus* was reared and also to the sex ratio. It was felt that the latter effect was tied in with the copulation rate. Christophers (19) found that both the number of settlements and of wheals increased from the fifth to the seventh day of emergence. Since practically all mosquitoes which settle do eventually bite, the settling rate may with sufficient accuracy be called the biting rate. As might be expected, the biting rate in a cage falls off with time as mosquitoes become engorged and retire to the sides of the cage. McCulloch & Waterhouse (85) observed a similar phenomenon in the field and suggested that the subjects in a field test should move about periodically from one spot to another.

The biting rate and the repellency of a given compound vary with dif-

ferent host factors. It has been known for some time that different subjects are susceptible to different degrees [McCulloch & Waterhouse (85); Travis & Smith (128)]. Attempts have usually been made in screening tests to control for this by such measures as paired tests on both arms or legs of a subject [Granett (56); Granett & French (57)], replications on different subjects, etc. The factor of host susceptibility has been evaluated most carefully in studies of dairy cattle repellents [Fryer *et al.* (47)]. Similar careful analyses are required with human hosts. Furthermore, since the repellent must work against host attractants, it is desirable that much more be learned of host attraction than now is known [cf. Laarman (77) for a complete discussion of host attraction].

McCulloch & Waterhouse (85) observed that there seemed to be a difference between the attractiveness of arms and legs not correlated with hairiness. On the other hand, Travis *et al.* (126) and some later workers felt that there were no such differences. Pijoan *et al.* (99) and Pijoan (98) stressed the very great importance of the condition of the host and the ambient environment in affecting repellent time. They showed that the greatest change was produced by dry heat. Thus, a change from 80°F. dry bulb and 70°F. wet bulb to 90°F. dry bulb and 70°F. wet bulb caused the protection time afforded by dimethyl phthalate to drop from 267 to 99 min. With increasing relative humidity, i.e., from 90°F. dry bulb and 70°F. wet bulb to 90°F. dry bulb and 80°F. wet bulb, there was little additional decrease (from 99 to 84 min.). The temperature of the host itself is certainly important because biting rate decreases on a cold arm [Christophers (19)]. Furthermore, Kasman *et al.* (71) found by correlating repellency with temperature that the shorter protection times on guinea pigs as compared to man could be explained in terms of the higher body temperature of the guinea pig.

Effective protection by a repellent varies not only with the behavior, etc. of the mosquito and the environmental conditions but also with loss and changes in the repellent itself as a result of host factors as well as environmental factors. The amount of repellent applied is very important [Christophers (19)]. Protection time and amount are not, however, directly proportional [Granett & Haynes (59)]. Sweating has an effect on repellent time attributable in part to its diluting action [Pijoan *et al.* (99); Jachowski & Pijoan (68); Starnes & Granett (119)]. Decrease in repellent time under simulated tropical conditions is partly attributable to sweat [Pijoan (98)]. However, Starnes & Granett (119) in some experiments which show that the effect of sweat may not be a dilution one only, found that a synthetic sweat diminished the repellency of Indalone but enhanced that of Crag Fly Repellent. According to Kasman *et al.* (71) the chief loss of repellency, at least of some repellents, is through skin absorption [cf. also Wiesmann & Lotmar (136)]. Tests of the rate of evaporation of dimethyl phthalate showed that the rate of loss by this route is much lower than the loss of protective action on the back of a guinea pig or on the human hand. It is argued that the loss

of protection must therefore be a result of hydrolysis or absorption through the skin. To test rates of skin absorption 0.25 ml. of 1-phenyl-2-hydroxypropanone-1 was placed on the shaved back of a guinea pig and the urine analyzed for excess benzoic acid, the presumed metabolic product. The yield represented 4.5 per cent of that theoretically available from oxidation of the original compound and indicates a significant loss by absorption.

Certainly in some cases breakdown of the repellent from chemical causes results in changes in protection time. Pijoan *et al.* (99) and Jachowski & Pijoan (68) were able to show, for example, that beta tetralol first became more effective as it oxidized and then later decreased in effectiveness.

Finally, the long effectiveness of repellents on cloth (days or weeks) as compared with that of the same repellents on skin points clearly toward the importance of absorption or breakdown as a limiting factor in the duration of effectiveness of skin repellents. It is probably as a result of many of the limiting factors which have been discussed and others compounded that protection time in the laboratory is almost always longer than in the field [Christophers (19); Travis & Smith (128); Starnes & Granett (119)].

Variability in techniques and poor choice of criteria of repellency have also contributed to the wide range in results observed by all. Laboratory and field tests as they have been conducted to date possess one virtue, a rapidity of screening. There has been very little uniformity and standardization from one experimenter to the next. Dosages per unit area are measured in drops [Pijoan *et al.* (99)], teaspoons [Travis (124); Travis & Smith (128)], cc. (most workers), or grams [Roadhouse (101)]. They may be applied at the rate of 1 cc. on the arm and 2 cc. on the leg [Granett (55)], 1 cc. per 100 sq. in. of skin [Granett & Haynes (59)], 1 cc. on the arm and 1.5 cc. on the leg [Travis *et al.* (126, 129); DeFoliart (21)], varied dosage [Christophers (19)], 0.5 to 0.8 cc. on hand and arm and 3 cc. on leg [McCulloch & Waterhouse (85)]. They may be applied full strength or as 25 per cent ethanol solutions [Travis (123); Applewhite & Smith (4); Applewhite & Cross (3); Altman & Smith (1)]. The criterion of skin repellency when mosquitoes are employed has usually been time to first, second, third, or fifth bite; for clothing repellents the criterion has been time to first bite, rate of biting, number of bites. With biting flies the criterion is sometimes time to first, second, third or fifth bite or the number of bites in a given time. With ticks and fleas the criterion is usually the number of animals in the treated area. For any given laboratory there has been extreme variability in results. This has been remarked upon by Granett (56), Pijoan (98), Linduska & Morton (82), and Travis (124).

Control and evaluation of variables.—The prototype of all tests and the first major attempt to standardize was the technique developed by Granett (55). His original test, a field test, consisted of exposing first an untreated arm or leg for 2 min. to determine the biting rate. Then an arm rubbed from wrist to elbow with 1 ml. of repellent or a leg rubbed from ankle to knee with 2 ml. was exposed continuously. The untreated control was exposed for 2

min. at 15 or 30 min. intervals to determine the biting rate based on the average of all counts. The criterion of repellency was the time to the first bite. Christophers (19) pointed out quite clearly the objection to this criterion. He noted that one is actually testing for two things (a) the essential effectiveness (intrinsic repellency) of the compound and (b) the duration of repellency. He advocated mapping and counting of wheals and pointed out that if this procedure were not feasible, even the simple contrast of biting rate on check and control arm is a better criterion than time to first bite. This point will be discussed further on in greater detail.

Variability could be reduced or at least statistical reliability of results could be ascertained. In place of human arm tests, tests with mice [Eddy & McGregor (36); Wiesmann & Lotmar (136)], with canaries [Wasicky *et al.* (133)], with guinea pigs [Wasicky *et al.* (133); Kasman *et al.* (71)], and with rabbits [Starnes & Granett (119)] have been tried. From the results obtained there is no reason to judge that these animals are inferior to man as test animals; and with respect to ease of manipulation, etc. they are superior. Toxicological screening tests are run on animals; there is no more reason why repellent tests should not be. Yet, like tests on man, these can serve only the purpose of quick gross screening. The many variables still exist.

Nowhere has this been better realized than in tests of repellents of cattle flies. The earliest methods are reviewed by Dethier (22). In 1947 Waterhouse adapted the indol plug method of Mackerras & Mackerras (87) for studying blowfly repellents. This consisted of soaking cotton plugs in 0.04 per cent indol, 2 per cent NH_4CO_3 , and 2.5 per cent ethanol and tying them on the fleece of living sheep. Of two plugs so tied, one (the control) was surrounded by a ring of paraffin oil and the other (the test) by a ring of the candidate repellent. An area of untreated fleece was left between the plug and the ring. The criterion of repellency was the number of egg batches laid on the plug. Two kinds of repellency were observed. Flies attracted to the plug either alighted at some distance from it and walked to it or landed inside the ring. In the first instance walking flies were repelled when they came to the ring (contact repellency). In the second case they were repelled only if the ring consisted of a volatile material (vapor repellency). Loeffler & Hoskins (83) tested the toxicity and repellency of certain organic compounds to the larvae of *Lucilia sericata* by allowing the larvae to form sinuses in an agar medium covered by raw wool. After treatment with test compounds this simulated wound was placed adjacent to an unsprayed sinus so that larvae could migrate if repelled. Repellency was determined by the number which emigrated.

Fryer *et al.* (47) made a thorough study of variability and experimental design. Two methods had previously been used, the whole-cow and the half-cow methods. There are basic disadvantages to both. In the former there is marked variation in the susceptibility (attractiveness) of each cow. Granett *et al.* (61) allowed for varying susceptibility of cows to *Stomoxys calcitrans*,

Siphona irritans, and *Tabanus* spp. by first obtaining a corrected normal count

$$\frac{\text{average number on checks during test}}{\text{average susceptibility count of checks (three or more counts before or after tests)}} \times \text{susceptibility of treated animals.}$$

The per cent repellency was derived from the expression

$$\frac{\text{corrected normal count} \times \text{treatment count}}{\text{corrected normal count}} \times 100.$$

In the half-cow method the fly's choice of which side on which to alight depends upon what is on the opposite side. Also, if the test material on one side kills flies, there will be fewer flies on the other side even if it is untreated. This could lead to the anomalous conclusion of no repellent being superior to a toxic repellent [cf. also Granett *et al.* (60)]. In order to evaluate the two methods Fryer *et al.* made comparable experiments with each. First, balanced groups were made on the basis of each cow's attractiveness. A latin square design was employed in the whole-cow method and a symmetrical pair design for the half-cow method. The raw data obtained showed a serious nonhomogeneity of variance among group counts, days, weather, etc. Because of their anormality the data must be transformed mathematically to a normal frequency distribution in order to be analyzed for significance. The most successful transformation was the reciprocal square root although a log transformation also seems to be satisfactory. Analyses of this sort demonstrate the superiority of the whole-cow method. For this method to be successful, however, one must first carefully obtain a measure of individual cow susceptibility (attractiveness), set up a balanced group, employ a latin square design, and make periodic counts. The analyses of Fryer *et al.* prove beyond all doubt that statistical methods must be employed in repellent research. This conclusion is most certainly applicable to tests with man, and much more reliable results could be obtained if more careful attention were given to the design of experiments.

Design of experiments.—As has already been pointed out, in designing a test for repellency one should first ask what information is being sought. If one hopes to relate repellency to chemical constitution, the problem must be broken down into its component parts before any useful analyses can be made. Tests with man and animals present an extremely complicated situation. Assuming for the moment that such variables as transient differences in insect behavior and biting rate, diurnal and individual differences in host attractiveness, etc. can be rigidly controlled, one is then confronted with (a) the intrinsic repellency of the compound to the insect, i.e., the chemical/physiological activity which affects the insect nervous system via the sensory system or otherwise and causes the insect to depart, (b) the change of effectiveness with concentration, i.e., whether or not the dosage-stimulation

curve has the same slope for all compounds (one should know the slope in order to ascertain which concentration of compound x is comparable to a given concentration of compound y), (c) differences in vapor pressure which cause changes in concentration as the test progresses and as the ambient temperature and the temperature of the host changes [Kasman *et al.* (71)], (d) changes in repellent concentration as a result of absorption by the skin [Wiesmann & Lotmar (136); Kasman *et al.* (71)], and (e) changes in concentration and constitution on the host as a result of sweat [Jachowski & Pijoan (68); Pijoan *et al.* (99); Starnes & Granett (119)].

Christophers (19) recognized some of these difficulties when he pointed out objections to the time to first bite as a criterion of repellency. He showed that in reality one was measuring two different factors, repellency and duration. The same objection holds with reference to time to first confirmed bite, or second, third, or fifth. Curiously enough, in tests with ticks, fleas, and cattle flies, numbers of arthropods are counted [e.g., Smith *et al.* (112); Smith & King (116)]. Actually there is no reason now why repellency should not be studied with tests analogous to dosage-mortality tests of insecticides.

Let us assume for the sake of simplicity that the repellent concentration remains constant in a test where a population of insects is exposed. There is every reason to believe that there is a normal distribution of thresholds in the population [Dethier & Chadwick (30); Dethier & Yost (33)]. The time to first bite or alightment would be a measure of a rise in threshold of the least sensitive individual attributable to adaptation or other changes. If the concentration did change (as in reality it does), the time to first bite would be a measure of a drop in effective concentration to a level of threshold of the least sensitive individual. Comparisons based on time to first or even fifth bite are based on the ends of a normal distribution curve, statistically the least significant portion and hence subject to great variability. To study intrinsic repellency with a view to comparing effective chemical structures, it would be better to compare the concentrations of different compounds that would repel 50 per cent of the population.

The first step toward answering this question demands a different type of test. In practically all present tests repellency is determined as the ability of a compound to overcome the natural attractiveness of a host. Whether the host is man, canaries [Wasicky *et al.* (133)], mice [Eddy & McGregor (36)], rabbits [Starnes & Granett (119)], guinea pigs [Kasman *et al.* (71)], or cattle (many), the variations in attractiveness and all additional phenomena introduced by the host mean that a repellent is being tested against a varying background which is very difficult to control and in many instances totally unknown. One can only agree with Hocking (65) that "no scientific approach to the problems of personal protection from biting flies (and all biting arthropods) is possible without an understanding both of the factors which attract these insects to their hosts, and those which stimulate them to feed." In the present absence of adequate information of this kind the test could be balanced against some controllable attractive force, e.g., temperature, light,

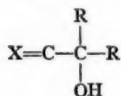
humidity, or gravity. The selection of the force should bear some relation to the attraction to hosts [cf. Laarman (77)].

This has been done with some success in certain cases. Although Smith & Gouck (115) found that tests in which ticks were allowed to cross treated bands were inconsistent and unsatisfactory, Granett & Sacktor (62) reported good results with comparable tests using the tick *Amblyomma americanum*. Linduska *et al.* (81) and Smith & Burnett (109) used treated and untreated cloth patches waved over jumping fleas in successful tests. Loeffler & Hoskins (83) in testing repellency for blowfly larvae used standard simulated wounds. Mackerras & Mackerras (87) and Waterhouse (134) used artificial attractants in the form of indol plugs. Most of the studies of repellency of DDT made use of inanimate surfaces [Kennedy (72); King & Gahan (76); Baranyovits (5); Brett & Rhoades (13); Dicke *et al.* (34); Rogoff (103); Hadaway & Barlow (63); Granett *et al.* (60)]. With the exception of the results reported by Christophers (19) and by Sarkaria & Brown (105), testing against a controlled attractant has not been done with mosquitoes.

Not only must this be done, but a distinction must be made between contact repellents and olfactory or vapor repellents. The difference has been noted by Christophers (19), Dethier (22), Waterhouse (134), and Sarkaria & Brown (105). There seems little reason why contact repellents for mosquitoes cannot be tested against some inanimate substrate as has been done with other insect repellents. Compare, for example, the methods used with cockroaches by Block (8) and Goodhue & Linnard (54) and the use of ants by Geigy & Utzinger (50). Vapor repellency can be tested against some standard controllable attractant as heat, moisture, or light. Christophers (19) and Sarkaria & Brown (105) have done this with *Aedes aegypti*, Dethier & Yost (33) and Dethier (26) with *Phormia regina*. Hughes (67) has tested the action of aliphatic compounds against species of *Glossina* without resorting to the use of any attractant. Kasman *et al.* (71) stated that an olfactometer does not satisfy the requirements of a test that would enable a correlation to be made between the results of the test and chemical groupings. They offer the following as objections: (a) it is not a rapid analytical method; (b) it does not take into account the essential function of a repellent as a barrier between the insect and the subject to be protected, and it defines only the effect of the repellent vapor without the surface from which that vapor must arise; (c) it does not define tactile repellency. Admittedly, present methods are slow and laborious. This is all the more reason for a concerted effort in alleviating the handicaps. The second objection fails to take cognizance of the importance of studying intrinsic repellency. The third objection requires no answer.

The chemical basis of repellency.—Considering the amount of effort which has been expended to date in the empirical testing of thousands of compounds, there has been disappointingly little advance in our understanding of the relation between chemical properties or structure and repellency. In 1925 Bunker & Hirschfelder (14) conducted a few poorly controlled field tests

with *Anopheles*, *Aedes*, *Psorophora*, and *Culex* mosquitoes in an attempt to relate repellency and chemical structure. Their tentative conclusions were that repellency is correlated with the presence of the oxygen atom, hydrocarbons being poorer repellents than alcohols, ketones, aldehydes, and esters. Not until 1953 was a further attempt in this direction made. Roadhouse (101) undertook an analysis of some of the data of Morton *et al.* (91) as a prelude to further critical testing. He selected for study the groups which Morton *et al.* had found especially effective in mass screening tests. His results confirmed the earlier conclusions of Bunker & Hirschfelder in showing that oxygen is an important constituent of the molecule. But, inasmuch as many compounds containing oxygen are not repellent, we must seek more subtle relationships than the mere presence or absence of a given element. Some of these subtleties are suggested by the findings of Roadhouse (101), Okazaki *et al.* (96), and Geigy & Utzinger (50). Roadhouse found that repellency is greatest when OH is once removed from unsaturation, e.g.,



and the 1,2 or 1,3 glycols or their dehydration products. Geigy & Utzinger (50) on the basis of biting tests with *Aedes aegypti* and orientation tests with the ant, *Formica rufa*, found that the most effective of the chemical groups tested were diethylamide, adjacent dimethyl with C—C, and adjacent diethylamide plus dimethyl groups.

Surveys of the lists of compounds which have been tested in the field or against animals in laboratories show that certain chemical categories are more often repellent than others under these conditions of testing. For example, Travis *et al.* (127) in summing up the work at Orlando for the period 1942 to 1947 found that the most effective compounds were esters, amides, imides, ethers, and alcohols. Roadhouse (101) found that aldehydes and ketones were poor, α -hydroxy esters with a boiling point range of 230° to 260°C. were effective, and cyclic mono-alcohols with a boiling point near 260°C. were also effective. He could demonstrate no difference between primary, secondary, and tertiary alcohols. It is difficult, however, to assess the significance of these conclusions because, of those compounds which are effective, we can only say that they possess the proper balance of properties which make for inherent repellency, long duration, and nontoxicity. For this reason, attempts to relate field performance to some molecular characteristic are bound to be less fruitful than a laboratory study. For example, an examination of repellent times for homologous series of phthalic acid esters, based on routine screening tests with *Aedes aegypti* and *Anopheles quadrimaculatus* revealed no uniform progression of effectiveness except for a rapid decrease above the three carbon compound [Haller (64)]. On the other hand, Dethier & Yost (33) and Dethier (26) in laboratory studies of the repellent

effect of aliphatic alcohols and aldehydes to blowflies found that within homologous series the molar concentrations necessary to cause rejection by 50 per cent of the population decreased progressively as chain length increased and that there was no rejection beyond the 12 carbon homologue. In terms of thermodynamic activities all intermediate members of each series are effective at the same concentration. Measured in molar concentrations the alcohols are more repellent than the aldehydes.

Similarly, in studies of the relation between vapor pressure and repellency, field and screening tests reveal the vapor pressure limits for effective field performance but shed little light on the relation between repellency and vapor pressure. Christophers (19) pointed out, for example, that an effective repellent must have a boiling point of approximately 280°C. to protect for 6 hr. but that effectiveness declines with boiling point above 350°C. With few exceptions substances with melting points above 37°C. are nonrepellent. Roadhouse (101) found that the best mono-alcohols were cyclic compounds with boiling points near 260°C.

Sarkaria & Brown (105) examined the question of boiling point (vapor pressure) by testing in an olfactometer the reaction of starved mosquitoes to 42 selected repellents. The vapor pressures at 25°C. of all compounds tested ranged from 0.1×10^{-3} mm. Hg to 660×10^{-3} . Dimethyl phthalate was 1.2×10^{-3} . The more volatile compounds tended to have the greatest repellency, but repellency is not just a measure of vapor pressure. Vapor repellency can vary independently of vapor pressure. Dimethyl phthalate, Indalone, and cyclohexanol have high vapor repellency and low volatility. This combination makes for long lasting repellents. The most repellent compounds were citronellal, *n*-hexyl mandelate, and 6-2-2. Dethier & Yost (33) also determined that there is no causal relationship between vapor pressure or boiling point and repellency [cf. also Ferguson & Pirie (37)].

As far as contact repellents are concerned, the most careful analytical work has been the laboratory studies of Frings (43) and Frings & O'Neal (46) of the stimulating efficiency of inorganic materials and similar studies by Dethier & Chadwick (29) of organic materials. This work has been reviewed in Roeder's *Insect Physiology* (102). However, stimulating effect alone is not sufficient to insure that a compound which is effective in the laboratory is equally effective in the field. Such is seldom the case. For example, Travis & Smith (128) found that repellents against *Stomoxys calcitrans* protected for a longer time in the laboratory than in the field. And Frings & Hamrum (45) found that substances which were irritating to *Culex* did not necessarily force the insects to move away [cf. also Wiesmann & Lotmar (136)]. Dethier (25) working with *Glossina* came to similar conclusions. Block (8) showed that some compounds which were rejected by cockroaches in taste tests were not necessarily effective against free cockroaches. But there are also some compounds (e.g., potassium thiocyanate) which repelled free cockroaches and yet were not unacceptable in taste tests.

The nature of repellency.—The relationship between repellency, stimulat-

ing effect, and irritating effects brings up the question of the repellent action of certain insecticides. When DDT first appeared it was believed that it had no repellent properties [Cameron (16); Buxton (15); Dethier (22)]. However, Gahan *et al.* (49) observed, probably for the first time, that DDT activated mosquitoes and caused them to leave buildings which had been sprayed. About the same time Wolcott (137) observed that wood which had been soaked for 10 min. in benzene solutions of DDT was immune to attacks by nymphs of the termite, *Cryptotermes brevis*, for as long as one year. Since mortality was negligible, Wolcott concluded that DDT was acting as a repellent. McCulloch & Waterhouse (85) observed that mosquitoes preferred not to rest on DDT-treated surfaces when others were available to them and that after feeding through treated surfaces the mosquitoes did not suffer high mortality. Gabaldon (48) showed that the disappearance of anophelines from sprayed huts was attributable to DDT and not to the solvent kerosene. King & Gahan (76) reported that DDT reduced the number of flies which would rest on grids. Muirhead-Thomson (92, 93) observed that DDT causes an increase in the activity of *Anopheles gambiae*, a change in the light response, and departure from treated huts without ensuing death, whereas benzene hexachloride was without noticeable repellent effect and killed mosquitoes in the huts. Bertram (7) had similar results with *A. minimus*; Wharton & Reid (135) with *A. maculatus*; Nair (95) with *A. maculatus* and *A. letifer*; and Baranyovits (5) with flies. Musgrave (94) reported a slight and temporary repellency to honey bees.

The first careful study of the nature of the reaction was that of Kennedy (72) with *Aedes aegypti* and *Anopheles maculipennis atroparvus*. He found that the presence of DDT on a surface (a) reduced the duration of resting periods, (b) increased the number of alightments, and (c) reduced the number of insects (*Anopheles*) settled at any one time. Quite clearly the DDT excited the insects to greater activity. Where they had a choice of treated and untreated papers, they rested nearly equally long if they had previously been exposed to DDT. They also moved preferentially toward light. In short, DDT produces an undirected excitation after a delay of some seconds or minutes and before a lethal dose is picked up. Firtos & DeJong (41) have called the DDT effect an "alarm reaction" and considered it, on no evidence, a respiratory excitation. It gives rise to events in the following sequence: (a) restlessness, (b) readjustment of sitting position, (c) change in normal light reaction, (d) flight followed by death. Hadaway & Barlow (63), studying the behavior of *Anopheles stephensi* on insecticidal deposits, found that the insects flew from all deposits in 2 to 4 min. but that death ensued only when the deposit consisted of particles less than 10 μ in diameter. DDT activation appears to be attributable to penetration of sense organs on tarsi during the contact period, and lethal effects are a result of dosages retained after pick-up. Smyth & Roys (117) have recently shown that DDT-sensitive houseflies spend more time on DDT-treated surfaces than on control surfaces but that a resistant strain does not. In neither strain was this result

obtained with DDE. It was suggested on the basis of convincing electrophysiological evidence that increased sensory input from tarsal chemoreceptors attributable to DDT gives an illusionary attractiveness.

This raises the question of just what a repellent is. Dethier (22) defined a repellent as "any stimulus which elicits an avoiding reaction." He pointed out that theoretically there may exist as many categories of repellents as there are classes of external stimuli. He recognized two primary divisions, physical and chemical, the latter consisting of olfactory (vapor) and gustatory (contact) repellents. Kennedy (72) recognized the same categories of stimuli which give rise to a repellent effect and then went on to make the important point that the reactions may take the form of merely random activity or of activity directed away from the source of stimulation. Reactions may be quick or slow to appear and weak or strong in character. He added, "... there is a widespread implicit assumption that repellency necessarily involves an immediate withdrawal from a treated surface." He suggested that "repellency" be confined to observed effects on distribution, that it not be used to describe the reactions of insects, that it involves reactions but is not a reaction, and that it is not necessarily of a normal sensory nature. "For practical purposes most workers would agree that a surface is repellent if insects are found to spend less time and so occur in smaller numbers on it than on other available and comparable surfaces." Rogoff (103) defined repellency as anything which reduces the numbers of insects on a treated surface. Utzinger (131) understood as repellents those substances which reduce insect bites by 10 per cent over an untreated control. Van Thiel (132) in speaking of the repellent effect of insecticides thought of it in terms of the total effect and behavioral aspects in the field. He recognized three main categories. (a) Insects are prevented from entering a hut. The effect may be "initial," i.e., attributable to the solvent; or "definite," i.e., attributable to the insecticide itself. (b) Insects do not remain in a hut. This repellency may be "complete," i.e., insects enter the hut, may bite, do not come into contact with the insecticide, leave. It may be "incomplete," i.e., the insects alight but are irritated and leave without biting ("primary") or after having bitten ("secondary"). Or they may bite first, then alight on a treated surface, become irritated, and leave ("tertiary"). (c) Insects never settle in a hut. Van Thiel concluded that in category (a) "initial" does occur, that in (b) "complete" could not be demonstrated, that (b) "incomplete" occurs, and that there are no grounds for assuming category (c) exists. He offered no explanation for categories (a) "definite" and (b) "complete," but agreed that (b) "incomplete" results from the alarming effect after DDT contact. He agreed with Firtos & DeJong (41) that this last should not be considered a repellent effect unless there is a clear understanding that it is a contact repellent effect.

From what we know of the action of chemicals on insects it is evident that some act as stimuli on the olfactory or gustatory sense and cause directed avoiding reactions which are more or less immediate. Such stimuli

would be true warning stimuli. Other compounds, like DDT, have some sort of an irritating effect upon an insect which may not necessarily be immediate but which causes undirected increased activity. Usually this would result in a decreased number of insects in the area causing the excitation. This would be positive orthokinesis of Fraenkel & Gunn (42). Obviously it need not work through the sensory system nor need its effect be immediate. It would be incorrect to confine "repellency" to observed effects on distribution, for the simple reason that such an application of the term is not informative and may actually be misleading. The absence of insects on a given surface could equally well be attributable to the attraction of some other surface as to the repellency of the first for any cause whatsoever. It seems best to realize that effects on distribution may be attributable to the insect actually avoiding an area, actually being attracted to some other area, or being excited to greater or lesser activity so that there is a biased distribution. In other words, a surface cannot be said to be repellent merely because it has fewer insects on it than on some other surface available at the time or a different time (e.g., paired and unpaired tests or half-cow method). It is more informative to recognize a compound as having produced a known effect. Thus, it would be best to recognize as chemical repellents those compounds which produce an immediate avoiding reaction. These repellents may be olfactory, i.e., vapor or distance repellents, and act either through olfactory or common chemical sense and cause a directional avoiding reaction (taxis).

They may be contact (gustatory) and cause under certain special conditions an immediate directional avoiding reaction (taxis). Then there are compounds which stir an insect to greater undirected activity either immediately or after some delay (DDT alarming reaction) and so cause the insect to leave a treated surface. It would seem best to reserve a special term for this type of phenomenon. It is very much like a kinesis. The question of whether these compounds react via sense organs or otherwise is not important as far as terminology is concerned.

Failure to eat a material has also been taken as a sign of repellency, but is not in itself proof of repellency. In plant feeding insects, for example, failure to feed may be a result of an absence of attractants to trigger the reaction; to olfactory repellents which prevent even the initiation of biting; to contact repellents which prevent feeding after sampling, or reduce it; and to toxic materials which, while they might not necessarily kill the insect, cause it to cease feeding or to move elsewhere. Thus the failure of termites to eat wood samples impregnated with DDT, even though mortality is negligible is not a sure sign of the repellency of DDT *sensu strictu* [Wolcott (137, 139 to 142)]. Protection against mites was at one time thought to be a repellent phenomenon [Madden *et al.* (88); Snyder & Morton (118)] but is now known usually to be attributable to paralysis of the mites; and the compounds now used for protection are termed miticides [King (74)]. In the case of moth-proofing agents where protection is measured in terms of reduced frass pro-

duction, loss of weight of cloth patches, etc. [Sweetman *et al.* (121)] effective compounds need not be repellents. The proprietary substance Mitin FF (a substituted urea containing dichlorodiphenyl ether) is a stomach poison for clothes moths but is said to be a repellent to carpet beetles. Eulan BL (dichloro-benzene-sulfon-methylamide) inhibits the action of digestive enzymes and renders wool indigestible. Eulan CN is not eaten by clothes moths and is said to be repellent but is a poison for carpet beetles. Eulan NK (dichloro-benzyl-triphenyl-phosphonium chloride) prevents digestion in clothes moths but is said to be a repellent for carpet beetles because it reduces the amount of feeding [Luttringhaus (84); cf. also Stoves (120); Bloomfield (9)].

Whether or not an insecticide is a repellent depends a great deal on the definition of repellency and the circumstances of the experiment [cf. Kennedy (72)]. Thus DDT was said to be repellent to termites [Wolcott (137)] and to clothes moths and carpet beetles [Luttringhaus (84)] on the basis of its prevention of feeding. Chamberlain & Hoskins (18) found that DDT was repellent to termites when a choice between a treated and an untreated surface was possible. In other situations repellency could not be demonstrated. On the basis of flies and mosquitoes leaving various DDT-treated surfaces, DDT was said to be repellent by some [Kennedy (72); Gabaldon (48); King & Gahan (76); Bertram (7); Field (38); Rogoff (103)] and an irritant by others [Gahan *et al.* (49); Muirhead-Thomson (92, 93); Firtos & DeJong (41); Wharton & Reid (135); Baranyovits (5); Nair (95); van Thiel (132)]. In terms of the reduction of the number of horseflies on a treated surface Howell (66) found that DDT was not especially repellent. Waterhouse (134) found that it did not discourage the landing of gravid females of *Lucilia cuprina* on sheep but did prevent oviposition. Under some conditions comparative counts of insects on treated and untreated surfaces have shown DDT to be an attractant [Kennedy (72); Smyth & Roys (117)]. In a Y-tube olfactometer Dicke *et al.* (34) found DDT to be definitely attractive.

Similar confusion arises as far as other insecticides are concerned. Brett & Rhoades (13) found that a barrier of dust and 5 per cent gamma isomer of benzene hexachloride was effective for as long as 72 hr. in causing the harvester ant to turn aside. Gammexane was found to be repellent to larvae of *Lucilia sericata* [Loeffler & Hoskins (83)] and to the housefly [DuChanois (35)]. On the basis of the comparative numbers of houseflies settled on grids King & Gahan (76) found that technical grade benzene hexachloride was more repellent than DDT, refined benzene hexachloride less repellent than DDT, and both wettable chlordane and methoxychlor nonrepellent or even slightly attractive. On the basis of fly counts on treated and untreated cattle Howell (66) found that benzene hexachloride, chlordane, and toxaphene were not repellent to horseflies. On surfaces treated with benzene hexachloride houseflies are generally quiescent and acquire a lethal dose before departing [Baranyovits (5)]. Rogoff (103), studying the reduction in numbers of greenhouse thrips on leaf disc preparations, found that DDT,

chlordan, parathion, heptachlor, and dimethyl phthalate were repellent and that gammexane was not repellent. In tests of the behavior of houseflies in a Y-tube olfactometer Dicke *et al.* (34) found that benzene hexachloride, chlordan, heptachlor, toxaphene, aldrin, lindane, DFDT, wettable powder of DDD, regular kerosene, and odorless kerosene were repellent while DDT and methoxychlor were attractive and dieldrin and technical grade DDD were neutral. Menke (90) found that toxaphene and parathion were only slightly repellent to alkali bees (*Nomia melanderi*). Hadaway & Barlow (63) found that *Anopheles stephensi* flew from surfaces treated with any DDT formulation in 2 to 4 min., and from chlordan, dieldrin, aldrin, and toxaphene after more than 30 min. Fresh deposits of benzene hexachloride activated and killed; but vapor from benzene hexachloride deposits did not disturb the mosquitoes if contact was impossible until after an advanced stage of intoxication was reached. With reference to pyrethrum, Findlay *et al.* (39) found that its presence on the skin reduced the number of tsetse flies alighting and biting. Johnson (69, 70) found that pyrethrum reduced the number of mosquitoes biting after they had momentarily landed and that lethane impregnated into clothing reduced biting by *Aedes* spp. Ribbands (100) found on the basis of night and day catches within huts that pyrethrum vapors and, to a lesser extent, lindane vapors deterred *Anopheles minimus* from entering sprayed huts. Paulini & Ricciardi (97) demonstrated what they interpreted as a repellent effect of pyrethrum on *Culex fatigans*. Laudani & Swank (78) demonstrated repellency of pyrethrum to several species of grain beetles.

It is certainly clear from the evidence at hand that repellency by whatever criterion it be judged is different from toxicity. There is no good reason a priori why toxicants should be repellent, e.g., CO is not repellent to man, nor Antu to some rats, boric acid to *Blattella*, formaldehyde to flies. Loeffler & Hoskins (83) found that there was no correlation between the ability of a compound to force larvae of *Lucilia sericata* to emigrate from treated wounds and its ability to kill. Chamberlain & Hoskins (18) found no correlation between repellent properties and toxic effects of series of compounds which were tested on the termites *Zootermopsis angusticollis* and *Z. nevadensis*. Sarkaria & Brown (105) studying the responses of female *Aedes aegypti* to vapors found that there was no correlation between repellency and knockdown (narcosis). Similar results were obtained with houseflies [Dicke *et al.* (34)]. In the case of the behavior of greenhouse thrips toward chlordan, DDT, parathion, heptachlor, and dimethyl phthalate there was an inverse relationship between toxicity and repellency [Rogoff (103)]. With isomers of benzene hexachloride there was no relation between the amount of time *A. stephensi* remained undisturbed on the surface and the per cent killed [Hadaway & Barlow (63)]. The insects rested more than 30 min. on the alpha and beta isomers and 1.3 min. on the delta isomer without being killed, but there was 100 per cent mortality from the gamma isomer where the resting time was 4.7 min.

Mode of action.—At the present time the most critical evidence suggests

that repellents act directly on chemosensory systems. Wiesmann & Lotmar (136) showed that *Musca* and *Stomoxys* deprived of their antennae, the principal loci of olfactory receptors, were unable to direct themselves away from vapor repellents. A similar result was obtained with species of *Glossina* by Dethier (25). Dethier (24) and Dethier & Yost (23) demonstrated with the blowfly, *Phormia regina*, that extirpation of all organs bearing olfactory receptors (antennae, palpi, and labellum) renders the flies insensitive to vapors which normally repel them. Furthermore, it was proved that failure to respond resulted from absence of the necessary receptors and not from interference with locomotion or induction of shock. Gradual reduction by surgery in the number of receptors leads to gradual increase in the concentration of vapor required to repel. Moreover, in the case of those compounds which are attractants at low concentrations and repellents at high, as for example *iso*-valeraldehyde [Dethier (26, 27); Dethier *et al.* (32)], removal of sensory areas mediating acceptance results in an inability to be repelled.

It is abundantly clear from the work of Frings (43), Frings & Frings (44), Frings & O'Neal (46), Chadwick & Dethier (17), Dethier & Chadwick (31), and Dethier (23) that contact repellents act upon specialized chemoreceptors which are not normally sensitive to vapors. Even DDT, when it acts as a repellent, affects chemoreceptors [Hadaway & Barlow (63); Smyth & Roys (117)].

Such receptors, located on the mouthparts and tarsi, are concerned with monitoring some aspect of feeding. One would expect that repellent compounds acting on these receptors would prevent feeding. Experiments with tethered insects bear out this expectation. And with free insects, contact repellents applied to mouthparts act similarly. However, as far as tarsal chemoreceptors are concerned, contact repellents may or may not prevent feeding depending upon other conditions, some of which are mentioned below. At the moment there is no good evidence that contact repellents on the legs invariably affect locomotor behavior. Insects do not always walk out of or avoid contact repellents although tethered insects frequently attempt to withdraw their feet from such solutions. Wiesmann & Lotmar (136) observed that some species of insects would be repelled by a chemical barrier while others would not. In special cases differences in response between free and fixed insects may be attributable to the experimental conditions under which the solutions are presented [cf., Block (8)].

On the other hand, there is some evidence that certain compounds in high concentrations act upon a common chemical sense [cf., Dethier & Chadwick (28)]. Roys (104) has shown by behavioral and electrophysiological methods that repellent vapors of such compounds as benzene act upon the leg (not known to bear olfactory receptors) and even on the isolated nerve cord of cockroaches. Slifer (108) has demonstrated that certain odorous materials held near the feet of grasshoppers provoke withdrawal of the appendages. Thus, while it is undoubtedly true that some vapor repellents can act upon senses other than the olfactory and gustatory, such repellents would act at lower concentrations and therefore, first, on the olfactory sense.

Some data illustrating this point have been collected from experiments with honey bees [Glynne Jones (52)]. Phenol and acetic acid act on the olfactory sense at low concentrations, but at higher concentrations the olfactory sense appears to cease operating while the common chemical sense takes over.

The practical problem of repellency is essentially a behavioral one, namely, to alter or interrupt a normal response operating through chemo-sensory pathways. One desires either to force an insect to depart from a given surface or to refrain from feeding through it. To be effective a compound must first possess inherent repellency, i.e., it must be capable of stimulating some sensory system other than that which mediates attraction or it must inhibit the system which mediates attraction. Second, since the response of the insect depends upon which sensory system has been stimulated and which reflex arcs are placed in operation, the repellent must act upon a system which has some influence on locomotion or feeding. As Frings & Hamrum (45) have shown, a mosquito may be intensely irritated by a compound touching the tarsi and still not step out of it. Furthermore, common field experience has shown that mosquitoes will stand in a spot of repellent and bite through a small unprotected area. In the case of *Phormia*, HCl vapor directed on the antennae will cause the fly to move away whereas HCl solution on the tarsi will not affect locomotor behavior but does under certain circumstances prevent feeding. If a fly is stimulated simultaneously by HCl on the tarsi and an attractive odor on the antennae, or HCl on the tarsi and sucrose on the mouthparts, feeding will not be prevented [see also Glynne Jones (52)].

It is clear that the nature of the response elicited by repellent compounds depends not only upon a variety of intrinsic biological factors such as age, state of nutrition, etc., but upon the concentration of the repellent, which sensory system it is stimulating, and whether and to what extent other sensory systems are being acted upon simultaneously by other stimuli.

In view of these considerations it would seem that a proper understanding of the mechanism of repellency depends upon knowledge of (a) the intrinsic repellency of a compound (stimulating efficiency), (b) the neural pathways through which the excitation is directed, and (c) the interaction of the various sensory and motor systems which determine the final response. Many compounds are eminently satisfactory from all of these points of view. The principal desideratum as far as repellents are concerned is to increase the duration of repellency. This is no longer primarily a problem of insect/repellent interaction. It is a question of absorption, dilution, alteration, etc. in the case of repellents applied to the skin and of ability to withstand wear and laundering in the case of repellents impregnated into clothing.

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SOIL INSECTS AND THEIR CONTROL¹

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"Some may feel that our soil insect problems are solved and that little remains to be done, but in my book we have only written the introduction to what should be one of the largest and best chapters in the history of economic entomology." This quotation from a recent address by Dr. G. C. Decker of Illinois is a concise appraisal of both the present status and the future possibilities of research on soil insects and their control.

Soil insects probably do at least some damage in every cultivated field throughout the world. Many times they mean the difference between a satisfactory crop and little or no return for the farmer's efforts. Any insect which, during its growing or feeding stages, lives either on or beneath the soil surface may be considered a soil insect. Many of them are not harmful to crops, but some rank among our most important pests.

Soil insects vary in both taxonomic relationships and habits (5, 33, 44, 66, 69, 83). Some of them like white grubs (*Phyllophaga* spp.) and wireworms (Elateridae) live persistently below the soil surface for long periods of time and feed on a wide variety of plants. Others like some of the cutworms (Phaeonidae) are nonpersistent forms and may not be present in great numbers in the same field for more than one or two years. Insects of these two groups, as a result of their ability to feed on numerous plants, usually occur in the fields before the crops are planted.

There is still another group of soil insects, including rootworms and root and seed maggots, which tend to be restricted in their choice of host plants and which usually invade a field only after a particular crop has been planted. A few species in this group, though commonly referred to as soil insects, do at least part of their damage by feeding above the ground. Corn rootworm (*Diabrotica longicornis* Say), flea beetles (*Epidrix* spp.), and sweetclover weevil (*Sitona cylindricollis* Fabr.) are examples.

Agricultural soils provide all the life requirements of a wide variety of insects. Both food and shelter are there for those adapted to take advantage of them. In addition soil provides refuge from natural enemies and buffers critical changes in temperature and moisture which might otherwise destroy them.

DAMAGE SYMPTOMS

All underground plant parts are attacked by soil insects, including the planted seed. Thus seeds, sprouts, stems, roots, bulbs, and tubers are at-

¹ The survey of the literature pertaining to this review was completed in June, 1955.

tacked. When the injury is slight or moderate, decay organisms often take over and complete the job. The cause of the damage is often overlooked or discovered too late to save the crop.

Loss of stand is a common symptom of soil insect damage to corn, soybeans, cereal grains, and many vegetable crops. The seed is destroyed or seriously damaged by wireworms, seed corn beetles, seed corn maggots, and certain others. Moderate seed and seedling damage shows up as stunted, yellowish plants with poor root systems. These symptoms are apparent early in the growing season, but it may take some digging and careful observation to find the cause.

Some of the other crop damages are just as drastic and easier to diagnose. Most farmers and gardeners are all too familiar with cutworms and their damage. Sod webworms do similar damage to corn and are often associated with cutworms in sod land. White grubs simply eat away the root systems, causing the plants to wilt and lodge or die. Corn rootworms destroy the roots, causing the plants to root-lodge during the next wind storm. If this happens while the plants are still growing they develop a characteristic "goose-neck" shape.

The general attitude toward soil insects in the past too often has been "out of sight, out of mind." It is easy to overlook the damage done by underground pests, especially when the damage is light or moderate and when excessive seeding rates are normally used. In addition the methods for controlling underground pests have not been too satisfactory until recently.

SOIL INSECTICIDES

The term "soil insecticide" has only recently come into common use. A soil insecticide is simply a chemical that is applied on or in the soil for the control of destructive insects there. Practically all soil insecticides also are used in other ways for insect control.

The use of insecticides on or in the soil is not new, but it had relatively little practical value prior to the introduction of the residual chlorinated hydrocarbons which followed DDT. The arsenicals are quite persistent in the soil, so much so that the soil in many old orchards is still poisoned with high levels of arsenic. Unfortunately arsenic in the soil tends to be more toxic to vegetation than it is to insects. The botanical insecticides are too unstable in the soil to be of any practical value there. Some very effective soil fumigants have been known for some time, but they are too costly to be of use under many circumstances.

Basically there are two different ways of using a soil insecticide. The first is to apply it on or in the soil in a spray, dust, or granular formulation. Usually it is worked into the soil in some way, rather than just spreading in on the surface and leaving it there. The other is to apply it as a coating or dressing on the seed, similar to the way seed-protectant fungicides are used. There are a number of variations in each of these procedures.

Research with modern soil insecticides is so new and the problems are so

varied that not all of the important questions can now be answered. However recommendations and suggestions can be made for preventing or reducing crop losses in practically every case where soil insects are troublesome.

SOURCES OF INFORMATION

A complete summary of the significant work on soil insects and their control would fill a book instead of a chapter. Our emphasis will be on recent developments, including practical applications, but attention will also be drawn to some of the more important early literature. The bibliography is intended to be a representative sampling of both the "classic" and modern literature, rather than a complete listing of all the important contributions.

A number of more or less general articles on soil insects and their control are now available. Gough's classic review of the literature on soil insecticides (34) marks the end of an era, since it came just before the advent of the chlorinated hydrocarbon insecticides. The work of Thomas (75) gives a unique coverage of an extensive early literature on the biology and control of wireworms.

Arnason & King (5) summarized a wealth of Canadian work and recognized the changes that were then under way. King (46) had previously published an excellent summary of the theory and practice of sampling soil insect populations. Boswell (8) contributed appropriately to the 1952 Yearbook of Agriculture of the United States Department of Agriculture. Lilly has authored three summaries or reviews dealing mainly with soil insect control on field crops, particularly corn (55, 56, 59). Bigger & Blanchard (7) have contributed important new leads and charted the course along which much additional work is needed. Publications dealing with pesticide-fertilizer mixtures include two by Jacob (40, 41), and one each by Farrar (23), Kulash (48), Lilly (58), and Woodle & Nettles (81).

CORN ROOTWORM CONTROL

The use of soil insecticides for the control of corn rootworms in the United States corn belt probably involves more treated acres and more pounds of insecticides than any other soil insect control program devoted essentially to one insect on one crop (58). Much of what is known about rootworm control with insecticides is applicable with only minor modifications to the control of other soil-infesting pests. Therefore we shall first summarize the present status of corn rootworm control and then associate it with the general field of soil insect control.

Aldrin, heptachlor and BHC are the leading corn rootworm insecticides. All are highly effective at low dosages, and reasonable in cost. Aldrin is most widely used at present (58), although BHC was the first to be found effective for this purpose (37, 64). Experimentally, dieldrin, lindane, endrin, isodrin and chlordane have all been used successfully. DDT is not effective.

What about formulations? Both powders and granular insecticides have been used in fertilizer mixtures, and liquid formulations have recently been

introduced for this purpose. Thorough mixing is always needed. Emulsifiable concentrates are used in both broadcast and planter-mounted sprayers.

Rootworm insecticides are used in dosages ranging from one-half to one pound of actual insecticide per acre. With aldrin and BHC the half-pound level usually has been just about as effective as a pound where they were compared. For the present one-half pound is suggested as the minimum and one pound as the maximum dosages.

How long will these treatments remain effective? We don't know for sure yet, but we have good evidence that under some conditions a pound per acre applied broadcast and promptly and thoroughly disked in gives corn rootworm control both the year of treatment and the following year. Lower dosages banded along the rows can hardly be expected to be effective more than one year.

The successful methods of applying soil insecticides for rootworm control on corn are listed as follows: (a) Use of starter fertilizer planter attachment for applying insecticide-fertilizer mixtures in place of ordinary starter fertilizer. No extra operations are involved where these mixtures are commercially available. Low dosage levels are adequate because the insecticide is concentrated in the root zone where rootworms and other stand-reducing insects do their greatest damage. (b) Planter-mounted sprayers which are designed to spray an insecticide mixture in a narrow band just behind the planter shoe at the time of planting. They do a good job of putting a prescribed dosage of chemical right where it will do the most good. Their cost is nominal and they give good service. (c) Broadcast spraying, using insecticides in the emulsion form in low-gallonage applications, and disking or plowing soon after applying to incorporate the insecticide into the soil. Excellent results are obtained with low-pressure spraying equipment. (BHC is recommended only for broadcast application.) (d) Broadcast application of insecticide-fertilizer mixtures or granular insecticides will accomplish the same result as broadcast spraying, provided the insecticide is properly worked into the soil.

Three other methods of application after the corn is up have not proven effective in controlling rootworms in the work to date. One was spray applications banded along the rows after the corn was up, either with or without subsequent cultivation to work the insecticide into the soil. The second was the use of insecticide-fertilizer mixes as bands along the rows with a deep-placement fertilizer applicator set at a depth of three inches and spaced about nine inches on both sides of the rows. The third was the side-dress application of insecticide-fertilizer mixtures at the time of the first cultivation.

The insecticides and methods of application described above for rootworm control are the major ones now in use for the control of other soil insects. However the insecticide dosages vary greatly with the insect, crop, and method of application. For example broadcast soil treatments seem to require about two pounds of a recommended insecticide for wireworm con-

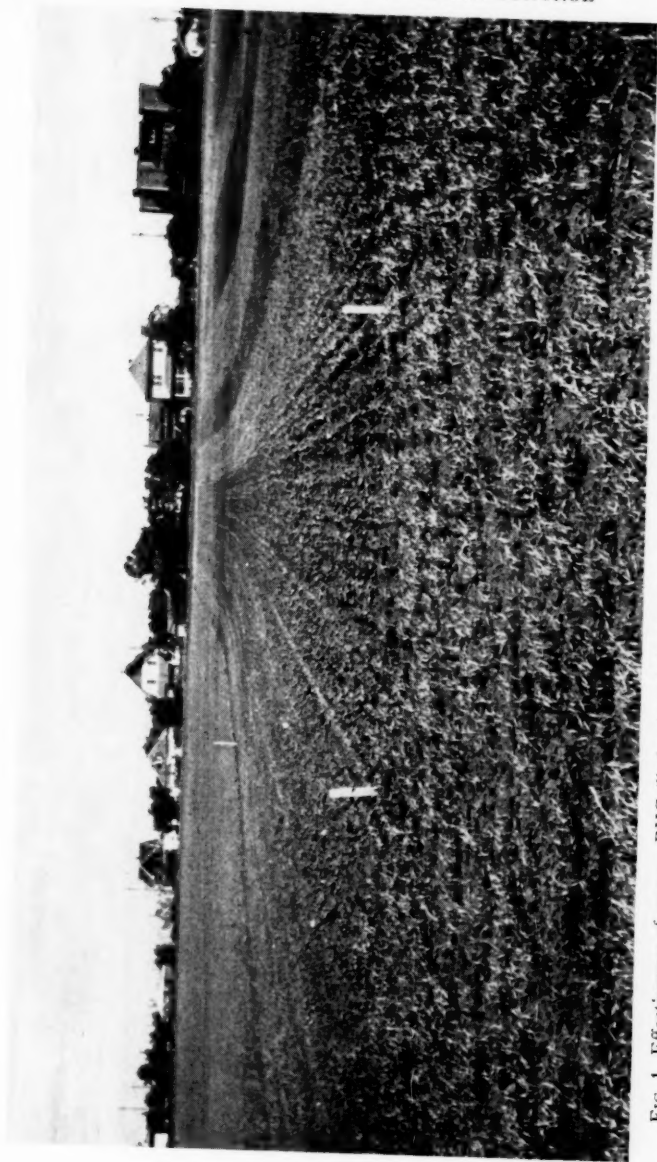


FIG. 1. Effectiveness of gamma BHC (lindane) seed dressings in preventing damage by the prairie grain wireworm, *Ctenicera acripennis destructor* (Br.), to wheat at Saskatoon, Sask., Canada. *Untreated strips*: bare, crop completely destroyed by wireworms; *treated strips*: normal stand; the seed dressings were applied to the seed at 1 oz. gamma BHC per acre. (Courtesy Canada Department of Agriculture.)



FIG. 2. (above) Control of onion maggot and smut in onions by in-row application of insecticide and fungicide on a granular carrier. Four untreated control rows in center between stakes; treated onions on both sides. (Courtesy Michigan State College.) (below) Control of wireworms in corn by an experimental application of heptachlor on No. 4 vermiculite in the planter trench. Two untreated control rows in center; treated corn on both sides. (Iowa State College photo.)



FIG. 3. Systemic insecticides show promise when used as seed treatments. (above) Cotton plant on left grown from seed treated experimentally with American Cyanamid Compound 12008; untreated control plant on the right. (Courtesy U. S. Department of Agriculture.) (below) Potato plant at left grown from tuber treated experimentally with American Cyanamid Compound 12009 on charcoal dust carrier; untreated control plant on the right. (Courtesy Michigan State College.)



FIG. 4. Effectiveness of soil insecticides in control of the corn rootworm, *Diabrotica longicornis* (Say). Six rows in center were untreated controls, while the plots on both sides were broadcast sprayed at the rate of one pound of actual insecticide per acre before planting. (Courtesy Burlington Hawk-Eye Gazette.)

tol, three pounds per acre for white grub control (59), and two applications, each at three pounds per acre, for control of the woolly apple aphid on nursery stock (14). A general rule is to use twice as much insecticide in a broadcast treatment as is applied in a row or band application.

It should be emphasized that these soil insecticide dosages apply primarily to mineral or upland soils. On peat and muck soils with their high organic matter contents these levels need to be increased to give comparable controls of the same insects. The best general rule we can give is to double the dosage commonly recommended for mineral soils when peat or muck fields require treatment.

Soil insecticides are used at surprisingly low dosages, even when maximum levels are employed. In round numbers the plow depth of an acre of ordinary soil, i.e., the top six or seven inches, weighs two million pounds. Thus it takes about two pounds of actual insecticide worked into the plow layer of an acre to give a concentration of one part per million. Many a farmer has wondered how he could possibly be doing any good when he was putting on such a ridiculously small amount of material to start with, and then diluting it as much as possible with the soil by plowing or disking.

Four methods of insecticide application not adapted for rootworm control are in use for other purposes. These are (a) insecticide seed dressings, (b) spray applications banded along the rows after the crops are up, (c) insecticides in transplanting water, and (d) band treatment of forage crop seedlings. Each of these will be considered briefly because they all fit into the general picture of soil insect control.

INSECTICIDE SEED TREATMENT

Seed treatment with insecticides is a challenging phase in the over-all story of soil insect control. The chief merits of seed treatment are that it is cheap and easy to use. Its disadvantages are that with row crops only a very low dosage of insecticide can be applied per acre, and its distribution in the soil is restricted to the immediate vicinity of the seed (22, 52, 55, 56, 71, 72).

The leading insecticides for seed treatment use are lindane and dieldrin. High-gamma BHC preparations have been extensively used on seed wheat in the Great Plains area of North America (5). Heptachlor, aldrin, EPN and Diazinon are other insecticides which may be worthy of further consideration. Parathion showed up well in early tests (17) but seems too hazardous for this use. Chlordane and DDT are of relatively little value (55).

Seed treatment formulations are almost invariably wettable powders. They may or may not be pre-mixed with a fungicide, but usually seed that is insecticide-treated also receives a fungicide (38). At least a small amount of sticker is usually desirable and methyl cellulose is the one in most common use. Insecticides dissolved in organic solvents or formulated in emulsifiable concentrates do not appear very promising for seed treating purposes (56, 72).

In the corn belt 25 per cent lindane wettable powder has been widely sold

for treating seed corn. Most of it was mixed in the planter box at 4 or 4½ ounces per bushel just before planting. In the irrigated areas of the West 75 per cent lindane is sometimes used. Dieldrin is now on the market for seed treating purposes, usually in dry formulations containing either 50 or 75 per cent of active insecticide. In combinations with thiram or captan it is present in various concentrations shown on the labels.

Farmer applications of insecticide seed treatments may be on the way out. For the first time in 1955 a large quantity of commercial field corn seed was treated with both fungicide and insecticide (dieldrin) when sold. This practice seems destined to grow. Seed treatment and direct soil applications are not truly competitive in most situations, and often they complement each other nicely.

In the corn belt seed treatment is primarily effective against seed corn maggot, seed corn beetles, and possibly Collembola. It helps check low infestations of wireworms, but usually is disappointing against heavy populations in most parts of the United States. In the South it is at least partially effective in reducing damage by the southern corn rootworm.

Seed treatment with the new systemic insecticides is still in the experimental stage, but it seems to hold some intriguing future possibilities (65). Health hazards to the people who treat and handle the seed, and toxic residues in feed and food crops, appear to be its most important limitations.

OTHER METHODS OF APPLICATION

In a few cases row or broadcast applications of insecticides after the crop is up are practical. The control of cutworms, sod webworms, or billbugs on corn are common examples (55). The time may come when applications made prior to or at the time of planting will largely eliminate the need for such postplanting treatments, but they still have an important place.

The use of insecticides in transplanting water is practical and effective for the protection of some transplanted crops (6). This procedure is economical and apparently is both safe and effective when properly carried out.

A recent publication from Ohio (77) describes a band placement method of applying insecticides for clover root borer control and reports good results with relatively low dosages.

BONUS VALUES FROM SOIL TREATMENT

Interesting and sometimes unexpected bonus values have resulted from the use of soil insecticides. Corn rootworm control not only increases actual yields but results in a greater percentage of the crop being harvested with less effort. Lodged corn slows machine picking and results in more corn being left in the field. Clogged pickers not only delay harvesting but sometimes result in serious accidents.

Many corn belt farmers have tried rootworm treatments in fields where rootworm injury was not expected just to see what would happen. Often the results far exceeded any reasonable expectations, presumably because some

other soil insects were controlled. Some have started a program of treating all their corn acreage as a form of crop insurance. This is not unreasonable when the cost of treatment amounts to about the price of a bushel of corn.

Soil treatment in at least one case appears to be the best means of checking an important plant disease through the control of its insect vector. Stewarts' disease of corn is largely transmitted in the corn belt by flea beetles feeding on the young plants. Experiments in Illinois in 1954 indicate that this disease was effectively controlled by surface applications of dieldrin sprays before the corn was up (60). Dosages in the range of three-fourths to one pound per acre were effective in preliminary tests.

MEASURING THE RESULTS OF SOIL TREATMENTS

Actual counts of soil insects in treated and untreated plots is at best a slow and tedious procedure (7, 9, 37, 45, 46, 57). In most cases soil samples have to be dug and carefully examined either in the field or in the laboratory. The populations are so variable that considerable replication is usually required to show significant differences. Some like rootworms have to be dissected out of the plant tissues. Others like *Collembola* are so small that they are overlooked without magnification, or are not present at the time of counting.

Traps are sometimes useful for obtaining quantitative differences in soil insect populations. A handful of spoiled corn buried to a depth of three to four inches and marked with a stake makes an ideal wireworm trap. However these traps also have to be dug and the insects counted. A number of mechanical devices have been used to sift insects from infested soil (50, 51, 53).

Several crop characteristics have been used where soil insecticides were being tested. In corn rootworm tests root lodging and grain yields have been used in addition to insect counts (9, 37, 57). For checking the general results of soil treatments on corn, plant stands, plant height, silking date, and ears lost by mechanical pickers have also been found useful (7).

Crop yield is always an important consideration from the farmer's standpoint. Some experts say that a field experiment is not completed unless yields are taken. Sometimes the quality of the harvested crop is affected by insect damage and should be measured along with the yield. Wireworm, flea beetle, and white grub damage to potatoes are excellent examples.

FARMER ACCEPTANCE OF SOIL INSECTICIDES

The real merit of a new agricultural practice is established by its acceptance by growers. The use of insecticide seed treatments on corn has increased steadily in the United States since initial tests in 1948 (17). A recent survey shows that an estimated 216,000 bushels of seed corn were treated with insecticides in Iowa in 1954 (59). At five acres per bushel of seed, this would amount to 1,080,000 acres, or about one acre out of every ten planted.

The popularity of direct application of insecticides to the soil is even more

impressive, because it involves far higher dosages and consequently much higher costs to growers. The extent to which this method has been used in the states of Nebraska, Iowa, and Illinois, is represented in Table I. The application of soil insecticides in Iowa increased from zero in 1950 to an estimated one acre of corn out of every 17 planted in 1954. However this practice started earlier in Nebraska, following the pioneering research carried out in that state (37, 64) and involves even a larger acreage.

TABLE I
ESTIMATED CORN ACREAGES TREATED WITH SOIL INSECTICIDES IN IOWA,
NEBRASKA, AND ILLINOIS, 1951 TO 1954*

State	Year	Number of Acres		Total
		Treated with Insecticide- Fertilizer Mixtures	Number of Acres Treated by Other Methods†	
Iowa	1951‡	10	15	25
	1952	15,000	10,000	25,000
	1953	200,000	125,000	325,000
	1954	362,500	237,500	600,000
Nebraska	1954	220,000	1,520,000	1,740,000
Illinois	1954	12,500	112,500	125,000

* Iowa estimates for 1952 to 1954 are based on surveys conducted by Dr. Harold Gunderson. The 1954 estimates for Nebraska and Illinois were obtained by personal communications with entomologists Roscoe Hill and Robert Roselle of Nebraska and John Bigger of Illinois.

† Mostly sprayed, either broadcast or banded on the rows with planter-mounted sprayers. This figure does not include the acreage planted with insecticide-treated seed.

‡ The 1951 acreage consisted only of Iowa State College experimental fields.

An amusing sidelight helps illustrate the effectiveness of soil insecticides and shows why growers accept them so readily. Several farmers have reported that when they planted corn at the usual rates after applying a soil insecticide they got stands that were too good! In other words the planting rates that gave them the desired stands with normal losses to soil insects gave them too many plants when the insects were controlled. "Complaints" of this nature are always good news.

There is a widespread impression that soil insect control is necessary and common only on a few field crops in the corn belt. Actually this is far from the truth, as may be seen in Table II. Among the less common crops in which soil insecticides play important protective roles are tobacco (6), sugar cane (61), peanuts (68), strawberries (21), narcissus (18), sweet potatoes (70), potatoes (63), root vegetables (46a, 72a), and nursery stock (14).

Very recently Jacob (41) has spent much time and effort in compiling a summary table of the pesticides used in pesticide-fertilizer mixtures. It includes the crops on which these mixtures are used, the pests they are intended to control, and the regions of the country in which the different combinations are used. Hence this compilation gives a good cross-section of the materials, crops, pests, and regions involved in this important method of soil insect control. It is included here by permission, supplemented with 1953 to 1954 use estimates from the same source (Table II).

PROBLEMS WITH SOIL INSECTICIDES

Problems and questions regarding soil insecticides and their use fall into three main categories. These are (a) What adverse effects do the insecticide have on beneficial animals, useful soil microorganisms and crops? (b) How can a fertilizer manufacturer meet all of the practical and legal requirements in making and selling insecticide-fertilizer mixtures? (c) How can a seedsman dispose of excess treated seed corn that cannot be disposed of as seed?

Earthworms are not controlled by soil insecticide dosages commonly recommended for the control of soil insects (63). In fact arsenate of lead at about 450 pounds per acre is recommended for earthworm control in preference to the chlorinated hydrocarbons in some states. The fate of beneficial predators and parasites in insecticide-treated soil is an important but largely unanswered question. When the results are successful the natural enemies theoretically are forced to seek food elsewhere. A heavy dosage of three pounds per acre of dieldrin over a large area apparently did not seriously affect populations of nabids, chrysopids, coccinellids, and blister beetle adults in Illinois in 1954 (60).

A number of papers relating to the effects of insecticides on beneficial microorganisms in the soil have been reviewed elsewhere (55). The general trend of these findings is definitely encouraging where the dosages are kept within reason. As a general precaution, over-dosing should be avoided. A recent paper on this subject is of considerable interest (26).

Most agricultural crops appear to show a fairly high tolerance to recommended dosages of soil insecticides (1, 2, 3, 10, 11, 13, 20, 24, 25, 30, 47, 49, 74, 82). Of the grain crops sorghums are among the most sensitive (13). Some varieties of vegetable crops are much more sensitive than other varieties of the same species (30). Unpublished work from Iowa showed no important adverse effects on field corn and soybeans from 50 pounds of aldrin, dieldrin, heptachlor, or lindane per acre, or from 100 pound dosages of chlordane, DDT, toxaphene, or methoxychlor.

The incorporation of insecticides into starter fertilizers created some important problems in the fertilizer industry. These have recently been considered in two papers by Jacob (40, 41), and one each by Kulash (48) and Lilly (58). The fact that practically every fertilizer producer in Iowa sold insecticide-fertilizer mixtures in 1955 indicates that the industry has met these challenges successfully.

TABLE II

SUMMARY OF FERTILIZER-PESTICIDE MIXTURES WITH RESPECT TO PRINCIPAL KINDS OF PESTICIDES, CROPS, AND PESTS*

Region and Estimated Short Tons Used 1953 to 1954†	Pesticide Used‡	Crop Treated‡	Pest Controlled‡
New England 950	Chlordane; DDT, lindane, 2,4-D	Turf, potato	Grubs, wireworms, weeds
Middle Atlantic 1,800	Chlordane, aldrin; DDT, dieldrin, 2,4-D	Potato, turf; corn	Wireworms, grubs, weeds; seed-corn maggot
South Atlantic 73,200	Chlordane, aldrin; DDT, dieldrin, heptachlor, toxaphene, 2,4-D	Corn, peanut potatoes, tobacco, vegetables; cotton, strawberry, turf	Corn rootworm, wireworms; cutworms, elongated flea beetle, grubs, maggots, mole crickets, sweetpotato weevil, weeds, white-fringed beetle
East North Central 6,700	Aldrin; chlordane, DDT, dieldrin, heptachlor, lindane, 2,4-D	Corn; onion, potato, strawberry, turf	Corn rootworm, wireworms; grubs, onion maggot, strawberry root weevil, weeds
West North Central 42,600	Aldrin; chlordane, dieldrin, heptachlor, 2,4-D	Corn; potato, turf, vegetables	Corn rootworm, wireworms; cabbage maggot, grubs, onion maggot, weeds
East South Central 1,200	DDT, chlordane; aldrin, heptachlor, 2,4-D	Corn; strawberry, tobacco, turf, vegetables	Corn rootworm, wireworms; ants, grubs, weeds, white-fringed beetle
West South Central 100	Aldrin, dieldrin, lindane, 2,4-D	Small grains, turf, vegetables	Grubs, maggots, weeds, wireworms
Mountain 1,450	DDT; aldrin, arsenicals, BHC, chlordane, heptachlor, toxaphene, 2,4-D	Potato, vegetables; alfalfa, small grains, sugar beet, turf	Wireworms; cutworms, grubs, maggots, weeds
Pacific 11,100	Aldrin; chlordane, DDT, heptachlor, IPC§, lindane, 2,4-D	Potato, strawberry; cane, mint, berries, hops, turf, vegetables	Wireworms, potato flea beetle, strawberry root weevil; grubs, spotted cucumber beetle, weeds
Territories 10,000	Aldrin	Sugarcane	White grub

* Adapted from Jacob (41), and used with permission of K. D. Jacob.

† 1953 to 1954 consumption estimates taken from the preceding table in same publication.

‡ For each region, the first group of items designates the major pesticide, crop, or pest.

§ Isopropyl N-phenyl carbamate.

|| Puerto Rico only.

Discard seed corn that is treated with an insecticide appears to be a "white elephant" in the seed industry. Whether or not the insecticide can be successfully removed at a reasonable cost is a pressing question. If not, insecticide-treated seed cannot be used in livestock feeds, and thus must be discarded and written off as loss.

CULTURAL AND BIOLOGICAL CONTROL

The question often arises whether soil insects can be controlled by cultural practices. To the extent that a cultural control method also constitutes a good general practice, it should be followed. Unfortunately it just does not work for many soil insects. For example, where meadow is included in the rotation in the corn belt, it is logical to follow it with corn. Yet in sod or heavily manured land, cutworms, wireworms, white grubs, webworms, billbugs, and seed maggots are most likely to give trouble.

Rotation for controlling corn rootworms is an interesting special case. Our common or northern corn rootworm in its larval stage is supposed to feed only on corn, and the eggs are laid in cornfields in the fall. Theoretically crop rotation should control it, but often it does not work out this way in actual practice. Heavy damage is often observed in old cornfields planted either to oats or soybeans the previous year. Two years with no corn always breaks the cycle and gives control, but this sort of rotation is not considered desirable by some farmers.

In summary, one should use the best farming practices known, including those for insect control where they seem justified. The stage has been reached where soil insect control is not just extra expense, but a definite part of good farming when control is needed.

Biological control of soil insects holds important possibilities that generally are not fully appreciated or utilized. The soil is a favorable environment for the survival of many pathogenic organisms, including bacteria, fungi, nematodes, and viruses, and for the development of some insect parasites. The biological control of the Japanese beetle in the eastern part of the United States is an outstanding demonstration of how an important economic pest can be controlled by its natural enemies. When the beetle was introduced into this area where none of its natural enemies occurred, it soon reached an epidemic status and became highly destructive to many crops and to turf areas. Within 20 years after beginning the colonization of its imported insect parasites, particularly *Tiphia vernalis* Rohwer, and of the bacterial diseases, including *Bacillus popilliae* Dutky and *B. lentimorbus* Dutky, the biological balance was established between the beetle and its natural enemies in many parts of the older infested area. The Japanese beetle is now only a minor pest in many areas where it was once highly destructive (79).

BIOLOGY AND ECOLOGY OF SOIL INSECTS

Biological and ecological studies of soil insects have been relatively neglected, particularly in recent years. What they lack in volume, however, is

compensated for in part by their quality. Of the more or less general references in this area, three of the Illinois reports by Forbes (27, 28, 29) head the list. Only by careful study can anyone fully appreciate the wealth of basic information they contain.

A number of more specialized early and modern contributions are listed in the bibliography. The pioneering work of Davis (16) on white grubs has been widely quoted. The work of Hayes (36) on the phytophagous scarabaeids of Kansas is truly a classic. Ritcher (67) studied this group of insects intensively from the taxonomic standpoint. Hammond in Canada has been concerned with long-term fluctuations in white grub numbers and with methods of forecasting them (35). Miner has added much to our knowledge of the biology of the prairie white grub (62).

Webster (78) carried out important investigations on corn rootworms. His work, along with that of Forbes cited above, is still the best original source of information on the biology of *Diabrotica longicornis*. However the southern corn rootworm has been intensively studied by several more recent investigators. Among these are Arant (4) and Isely (39). This insect is a major pest of peanuts, and only recently has it been efficiently controlled on this crop (68).

Two excellent general references on cutworms are listed. These are by Crumb (15) and Walkden (76), respectively. The pale western cutworm has been the subject of a number of investigations, including those of Cook (12) and Jacobson and co-workers (42, 43).

Wireworm studies prior to 1940 were well summarized by Thomas (75). One of the most comprehensive studies of an individual wireworm species is that of Stone (73). Wireworms and many other soil insects live largely beneath the soil where their normal reactions and activities are not subject to direct observation. This problem has been solved in part by tagging wireworms with radioactive tracers and following their reactions with Geiger count-rate meters (31, 32). The possibilities in this line of research have barely been touched.

Another "new" technique appears to have a practical application in the study of food habits of predators, particularly those which feed on soil insects. It is a precipitin test basically similar to those now used in medicine (19). Extracts from the pest species serving as prey are used to produce antibodies in rabbits. The rabbit serum is then used for precipitin tests with smears of predaceous insects collected in the field.

WHAT DOES THE FUTURE HOLD?

The rapid acceptance of soil insecticides to date, along with their economy and apparent lack of serious hazards or shortcomings, indicate that their practical utilization is still in its infancy. Certainly their use will grow where it is still sharply on the increase, and there is every reason to believe that other important applications are still to be discovered.

The persistent chlorinated hydrocarbons appear to be uniquely well

adapted for soil use. The recommended ones are highly effective for relatively long periods of time and are safe and economical at the recommended dosages (54, 59, 80). A second group worth watching are the new organic phosphate compounds which have a systemic action when used as seed treatments. Their hazards to the people who handle them, and the possibility of undesirable residues in feed and food crops, are important considerations which must be settled before they can be advocated for general use.

Further fundamental studies on the biology, ecology, and taxonomy of soil insects are needed. Most investigators will be tempted to ignore such possibilities in favor of insecticide studies expected to pay quick dividends in the form of practical utilization. Only when the gamuts of pests, crops, and materials have been much more fully explored may these basic but less spectacular lines of investigation be expected to receive the consideration they merit.

Anyone who has had much contact with corn culture can sympathize with the clever pioneer farmer who put into verse the reasons why he planted four kernels of corn for every plant he wanted:

"One for the maggot
One for the crow
One for the cutworm
and one to grow"

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STORED PRODUCT ENTOMOLOGY¹

(THE ASSESSMENT AND REDUCTION OF LOSSES CAUSED BY INSECTS TO STORED FOODSTUFFS)

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Discussions on the increasing world population and the consequent increasing food requirements focus almost all the attention upon food production and scarcely any upon the significant contribution which could be made by food conservation, namely, utilizing to the full the harvest won with such cost and difficulty. Whereas agricultural entomology has long received recognition and funds, stored product entomology has not. Too often it has been considered, in most parts of the world, the spare time job of the agricultural entomologist who, preoccupied with the problems of cultivation of crops, has had no effort to spare for safeguarding the harvest during storage and sometimes has lacked the necessary specialized knowledge. Doubtless, the importance of stored product entomology has not been appreciated largely because the damage done is of an insidious nature and is often not detected until the commodity is about to be sold or consumed. Moreover, with so few workers on this subject, the information on losses caused by insect infestation of goods in store is scanty, scattered, and poorly publicized. The author therefore intends in the first part of this review, to summarize information on the losses referred to in recent literature; and, in the second part, to consider briefly the measures now available to combat these losses. Since nearly all assessments of loss refer to foodstuffs, only these will be considered here and, as will be seen, most have to do with grains or pulses.

There are important differences between losses sustained from insect infestation during growth and during storage of crops. In agriculture, the effects of infestation are often visually striking and the cause must be dealt with by the farmer or he bears the loss; occasionally, however, early infestation in a field crop is partially or more than compensated for by increased yield from the surviving plants. With stored products, the harvest is gathered in a relatively small volume, say 10 to 20 tons of wheat from 20 to 25 acres of ground, and is generally stored in sacks or bins so that, except in the extreme, infestation is not conspicuous. Moreover, no true compensatory change can occur, although part of the loss may be masked by a gain in weight from increased moisture content and by the weight of the residual insects inside and outside the grains, together with their frass. Infestation, especially in cooler climates, may take several months to develop to a serious level, and the losses may be shared as a result of sales among handlers of

¹ The survey of the literature pertaining to this review was completed in May, 1955.

the products; at the same time, responsibility for treatment may be passed on. Finally, in agriculture the losses are virtual in so far as they can usually be assessed only against estimates of what might have been produced, whereas losses from stored products are real, the starting material being gathered at harvest and including in its value all the earlier expenditure of materials and labour for cultivating and fertilizing the soil, planting, growing, and harvesting.

ESTIMATES OF LOSSES

Estimates of losses may, for convenience, be divided into two categories (a) the general estimate, or the informed guesswork of the expert, and (b) the experimental estimate based on some actual measurements, however crude.

General estimates of loss.—The conference called in London by the Food and Agriculture Organization of the United Nations in 1947 showed that the delegates were fully aware of the damage done by insects to stored products, but their pleas and resolutions (1) have largely fallen on the stony ground of administration and finance. Some "seed" is undoubtedly germinating slowly as shown by the setting up of a Stored Products Working Party by the European Plant Protection Organization (2), but the seedlings badly need strong support.

The Food and Agriculture Organization has published an estimate of 5 per cent loss annually through insect infestation of all harvested cereals, peas, beans, and oilseeds, representing one-half the quantity of these products entering into world trade (3). The Food and Agriculture Organization considered this a conservative estimate because most loss occurs in tropical and sub-tropical countries, especially in under-developed areas, where information on losses is very largely lacking.

A Working Party of the United Nations (4) made an on-the-spot study in 1948 to 1949 of the losses sustained in Latin America and, in consultation with local authorities, arrived at the following figures: (a) In El Salvador and Guatemala, insects and fungi cause losses of approximately 25 per cent of harvested maize, rice, and pulses. (b) In Honduras, losses of maize are 50 per cent, and in Nicaragua 30 per cent. (c) In Costa Rica, losses of harvested cereals run at 45 per cent in spite of Government efforts at pest control; in addition, there were lost through infestation in storage during 1944 to 1948, 325,000 United States dollars worth of imported grain and, between December, 1946 and September, 1948, more than 100,000 United States dollars worth of locally bought pulses. (d) Ecuador incurred a total loss in 1947 and 1948 of over two million pounds of United States and Canadian flour. (e) In 1947, Venezuela lost 16 million United States dollars worth of maize, pulses, rice, coffee, and sesame, 82 per cent of this loss being ascribed to insects. Colombia similarly lost 11,400,000 United States dollars worth. (f) In Haiti, annual losses of stored wheat, maize, rice, and oats are estimated at about 47 per cent. (g) Even in a more temperate country like Chile, 38

million United States dollars worth were lost from the 1947 to 1948 crop, and Uruguayan losses were officially put at 14 per cent of the cereals harvested.

A few general estimates are available for other tropical and sub-tropical parts. Coyne (5) mentions that one million tons of stored grains are lost annually in India, over half being wheat and rice. Cotton (6) estimated that, in the Deep South of the United States, maize may lose up to 9 per cent in weight per month of storage. This accords with Eden's (7) assessment that often as much as 50 per cent of the maize stored through the summer on Alabama farms is destroyed by stored grain insects. He also estimated that the annual loss in Alabama during 1948 to 1952 was 25 per cent, or over 11 million bushels, of maize. In Northern Nigeria, Jepson (8) found bean weevils (*Bruchidae*) to be a serious drain on the food reserves, infestations of 50 per cent being commonly seen on cowpeas in the later part of the season. Bean weevils are also said by Toledo (9) to damage more than 20 per cent of the two million sacks of beans produced annually in Brazil.

In Germany, Zacher (10) has estimated that the loss from the granary weevil, *Sitophilus granarius* (Linnaeus), alone was about 100 million RM in 1933, an estimate later confirmed officially. Losses from the same insect in 1949 have been reported by Frey (11) as 2 1/2 per cent of the harvest, or 255,000 tons worth 71.4 million DM.

In the United States, Fairfield (12) considered in 1943 that the total annual loss of foodstuffs during growth and storage approximated to 2,000 million dollars. Of this, she attributed 300 million dollars to pests of stored cereals, including 60 million dollars for damage by the rice weevil, *Sitophilus oryzae* (Linnaeus). By 1951 Bishopp (13) had revised these estimates, largely no doubt because of increases in commodity prices, to 4,000 million dollars as the annual loss of foodstuffs, of which insect damage to stored cereals accounted for 600 million dollars. Haeussler (14) published similar figures in 1952, giving the actual amount of grain lost annually as 300 million bushels valued at 500 million dollars at 1951 prices. He also mentions an estimated annual loss of 150 million dollars between 1940 and 1944 from insect attack on processed foods and packaged goods. Cotton (6) has come to the conclusion that a 10 per cent weight loss may be suffered by grain stored during one season in the Great Plains region.

In his book on crop losses Ordish (15) scarcely mentions losses after harvest, perhaps because he could obtain so little information, but the figures in his Table I relating to agricultural losses from pests and diseases in the United States in 1937 and 1939 may be used to make the comparison given in Table I below.

Stored products are second highest in losses, yet a very poor fourth in expenditure on control measures. There is a need for more expenditure on research to extend the range of preventive and control methods and particularly to educate all concerned in the sequence of commercial handling from growers to consumers in the application and economics of this branch

TABLE I

LOSSES FROM INSECT PESTS IN THE UNITED STATES IN 1937 AND 1939

	Value of produce destroyed (a)	Cost of control (b)	b/a
	\$ '000	\$ '000	%
Agric. and hort. crops and farm animals	653,340	80,619	12.3
Man	261,775	47,914	18.3
Stored products	363,485	7,394	2.0
Forests	162,000	25,000	15.4

of pest control. Great savings of foodstuffs can be made by the application of relatively simple and cheap measures of hygiene and insecticidal treatment.

Experimental estimates of loss.—There can be no doubt that it will ultimately be on the basis of experimental estimates that a true idea of the real importance of losses attributable to insect infestation of stored products will ultimately be built up. Instances are at present few and field experimental work must be encouraged to increase their number. Valuable information could frequently be obtained by measuring the losses sustained by untreated controls in experiments. The experiments might also often be slightly modified in design to permit comparison of the loss in terms of money with the cost of the control measures to prevent all or part of it (20, 29). In passing, a point might be made of the real difficulty in some countries of persuading the nonscientist that there is an experimental necessity for control piles of grain, etc. in which insect infestation shall be allowed to run its natural course until a clear-cut result is evident.

Fortunately, the days are gone when infestations were transported about the world like that quoted by Cotton & Gray (16) when 1 3/4 tons of weevils were screened from 145 tons of maize. Similarly, means are available to prevent such losses as the equivalent of 2,200 bushels a day from a stock of 3 1/2 million tons of wheat stored in Australia for 3 years during the first world war (3). Nevertheless, Freeman (17) instances a loss of £10,000 in value suffered in 1948 by a cargo of £208,000 worth of Argentine maize which, during shipment to the United Kingdom, heated because of insect infestation.

Most of the information gained from experimental work or direct investigation refers to maize, but a few figures have been found for wheat, rice, sorghum, beans, and groundnuts (peanuts).

Harris (18) has referred to the experimental protection in Uganda of bagged seed maize of which 1 per cent of the grains showed damage at the start and 91 per cent in the untreated control six months later. Michelmores

(19), reporting later work in the same territory, stated that two bags of maize used as controls in an insecticide test showed 81 and 100 per cent respectively of damaged grains after seven months storage. He also mentioned that a random sample from a large bulk of bagged maize had 35 per cent of the grains damaged after six months in store. In an experiment of Le Pelley & Kockum (20) in Kenya, bags of fumigated maize placed in an infested store were shown by sampling four months later to contain over 32,000 adult *S. oryzae* per 100 kg. of the grain. More recently, Kockum (21) found 22.7 per cent less weight of maize shelled out from untreated cobs stored in cribs for six months compared with cobs treated with an insecticidal dust containing gamma-BHC. Control bags of "trade maize" in experiments by Cockbill (22) during five summer months in Southern Rhodesia showed an increase of 28.4 per cent in the numbers of damaged grains, which was reflected as a net loss in weight of 8.8 per cent. In top bags, where internal heating of the stack imposed no restriction on insect breeding, the corresponding figures were 56.7 and 19.0 per cent.

Hall (23) has very recently added to our information on losses a number of instances recorded during visits to British tropical territories. These are too numerous to itemize in this review, but one example (his case no. 2) may be cited regarding loss from maize stored for 12 months in bags without control measures. The apparent loss in weight was 3 to 4 per cent but, after allowance was made (by sampling) for insects and their frass, the true loss in weight was nearer 14 per cent. Even taking loss at the gross level of 3 to 4 per cent, the estimated loss of stored maize in the territory was 53,000 to 63,000 bags, each of 200 lb. Two years later, by adoption of tighter stacking and lindane dusting of the bags, the loss was cut by about 50 per cent (case no. 4) with an estimated saving of some 30,000 bags of maize per annum. An important point here is the saving which can be brought about by the application of measures which fall short of giving 100 per cent control. There is often a tendency to decry treatments which fail to give or cannot be expected to give complete control.

A final example of maize infestation comes from the United States where Eden (24) found from two years experiments in Alabama that shelled maize showed a mean increase in percentage of grains attacked by the rice weevil from 5.1 to 86.1 during 9 months crib storage.

Because of observations by the Grain Marketing Board of Southern Rhodesia that Kaffir corn (*Sorghum vulgare*) was subject to severe losses from insect damage during storage, Cockbill (22) experimented with various insecticidal treatments on small stacks of the bagged grain. During five months storage, however, the untreated sorghum suffered only 1.3 per cent net weight loss. This contrasts with other results. Lefèvre (25) describes a similar experiment in the Belgian Congo on the protection of sorghum against *S. oryzae*, in which four sacks of untreated grain showed 8 per cent of damaged grains three months after harvest rising to 85 per cent two months later; after 9 months in storage more than 94 per cent of the grains were

holed. Similarly, Nasir (26) kept clean sorghum in an infested store for 6 1/2 months and found 50 to 70 per cent of the grain damaged. In an experiment including two 40 kg. lots of untreated sorghum stored in sacks for 11 months, Harris (27) in Tanganyika recorded a weight loss of 19.7 per cent which increased to 23 per cent when insect waste and hollow grains were removed, but apparently without allowance for insects within grains. In the same paper Harris has published interesting figures (Table II) on the infestation in samples of the main native crops obtained from a local bazaar in Tanganyika. Although he points out that these losses may be maximal, they show a serious wastage of native foodstuffs through uncontrolled insect infestation.

In India, Pingale (28) found that there was an increase in the numbers of damaged kernels from 2.7 per cent to 48.5 per cent when wheat was stored in untreated jute bags for five months. Baeta Neves (29) has gathered information about losses caused to stored foodstuffs in Portugal and the costs of treatment. On wheat he reports that, early in 1950, 10,000 kg. in store lost 4.6 per cent by weight in 45 days because of weevil attack. Moreover, some 7.5 per cent by weight of flour examined by analysis of samples in 1952 was found infested and unfit for human consumption. In the United States wheat in wooden farm bins in Kansas was found by White (30) to show an increase of infestation from 4.6 to 41.4 insects per kg. during storage from July to September.

In the rice-growing areas of the United States rough rice, according to Balzer & Cotton (31), can lose up to 10.7 per cent by weight (corrected for moisture content changes) during storage for 12 months. They point out that insect infestation leads to a considerable increase in the proportion of broken grains during milling and that there was in 1947 a 40 per cent price differential between whole kernels and broken. Hall (23) reports an estimated weight loss of 4 to 5 per cent from rough rice stored for 12 months in West Africa which became a true loss of 14 to 15 per cent by weight when allowance was made for residual insects and frass.

Stored peas, cowpeas, and beans are very susceptible to insect damage in tropical countries. In the Belgian Congo, Lefèvre (32) found 48 to 70 per cent of beans damaged during storage for eight months after harvest and in a later paper (25) reported 93 per cent of beans holed by bruchids when stored in sacks for 12 months, by which time the beans were worth only 7 per cent of their initial value. In Southern Rhodesia, Cockbill (22) recorded a mean net loss in weight of 13.3 per cent during 5 months storage, the number of damaged beans having increased by 29.5 per cent.

Finally, to turn to groundnuts, Howe (33) made a detailed investigation of losses attributable to insect infestation in Nigeria and reported an average net loss of about 4.5 per cent during 12 months in store. He points out that in normal practice most of this is undetected because of the insect bodies, excrement, etc., remaining in the sack and because of a gain in moisture content of the nuts during transit to the port for shipment. Similar

TABLE II

INSECT DAMAGE IN PRINCIPAL NATIVE CROPS IN A BAZAAR IN TANGANYIKA

Crop	Seeds damaged (per cent)	Estimated loss in weight (per cent)
Sorghum	98	46
Cowpea	100	41
Haricot bean	46	3
Pigeon-pea	6	1

figures are given in a publication (34) of the United Kingdom Colonial Office, where it is added that *Tribolium* infestation of half and broken nuts can increase the free fatty acid content of the oil during 12 months storage from 3.1 per cent to 13.4 per cent; the loss of edible oil is about double the acid value. The same publication mentions as an ancillary loss, that some 80,000 bags are lost annually in Northern Nigeria by *Trogoderma* damage in the groundnut storage pyramids. Hall (35) has made an interesting analysis of damage in samples of the 1952 to 1953 crop of Gambian groundnuts and concludes that 2.4 to 16 per cent (average 5 per cent) of the nuts are damaged by insects after harvest, resulting in a 3 per cent loss in weight of the crop of kernels. On a harvest of 55,000 tons this represents a loss of 1650 tons valued at £90,000. Baeta Neves (29) has been able to determine that nearly 21,000 tons of groundnuts imported into Portugal from Guinea in 1951 had lost 5.93 per cent by weight through insect damage (*Pachymerus*, *Tribolium*, etc.) and that the losses in all groundnut imports were 6.62 per cent. This author also gives a little information about a wide variety of other products.

All the experimentally determined losses cited above refer to beetles. Moths and their larvae, e.g., *Sitotroga cerealella* and *Ephestia* spp., must also be responsible for great destruction of stored foodstuffs, but no measurements seem to have been made apart from Freeman's (36) report that 3,000 lb. of germ were eaten from 500 tons of wheat in two years by *Ephestia elutella* and Baeta Neves's estimate (29) that 150 escudos has to be spent on the treatment of each ton of figs exported from Portugal, to ensure freedom from active infestation by *Ephestia cautella*. Mites, too, are often injurious, and the little that is known about their depredations is summarized by Solomon (37).

Storage losses of the magnitude mentioned in this whole section on "Estimates of Losses" are often of the same order as those sustained during cultivation of the crop. They are a severe burden on the economy and welfare of the countries concerned and form a major contributory cause of the low levels of subsistence in many of these countries. Freeman (17) has analyzed the complexity of ways in which damage and loss may be sustained

and rightly points out that account must be taken of the variation of standards in different parts of the world. In undeveloped countries the peasant cultivator does not look too closely at the insects in his food, especially if he has nothing else to eat. Nevertheless, insect infestation may well cause stomach disorders in parts of Africa when, as recorded by Hall (23), samples of maize meal for native consumption showed up to 20,000 insect fragments and up to 20 rodent hairs per 25 g. According to Harris *et al.* (38), although determined by a different method of milling, an average of 12 1/2 fragments equals one whole insect. Assuming the weight of an insect to be 2 mg., it can be determined that some 13 per cent of the weight of the maize meal was in the form of insect remains, irrespective of any allowance for excrement.

OBSERVATIONS AND ASSESSMENT OF LOSSES

Although the figures assembled above are impressive, they would be even more so if the methods of assessment were less diverse and therefore more comparable. Losses have been estimated in monetary value, reduction in weight (uncorrected, partially corrected, or fully corrected for moisture changes, frass, etc.), numbers of damaged grains, increase in numbers of insects, and so on. The possibility of contributing data on losses should be borne in mind by every stored product entomologist planning small or large tests under practical conditions of storage and handling, but clearly the resulting data should be directly comparable with others. More use should be made of control piles for loss assessment and of random samples taken from cribs, local markets, or bulk piles in normal storage.

Whatever may be the most suitable measure of damage in a particular experiment, the author urges that an attempt should always be made to estimate the corrected weight loss. For example, the percentage of holed beans may be the desirable criterion in an experiment but samples of, say, 100 sound and 100 holed beans could be weighed, thus allowing conversion to uncorrected weight loss. The 100 damaged beans could then be opened, cleaned of internal insects and dust, and reweighed to give the corrected weight loss, assuming no change in moisture content. Knowledge of the current price of the commodity would permit estimation of the monetary loss. Observations on additional aspects of loss such as down-grading, increase of acidity, or loss of special nutritive constituents, could always be appended. Laboratory investigations, in rather more detail than those of Pingale (39), should enable useful tables to be constructed relating the percentage of damaged seeds to uncorrected and corrected weight losses, loss of germination, etc., for different products and different insect species.

Assessment of losses as a result of insect infestation of processed and packed foods is much more complex and would need the close co-operation of the trades concerned in gathering and analyzing data. Loss of reputation is often of far greater importance than the loss of product, but the difficulty of assessing the former need not be stressed.

A totally different kind of loss is that occasioned by the need for routine

treatment of commodities, whether infested or not, because, in the absence of treatment, serious infestations would rapidly build up in a high proportion of parcels. Much money is spent on preventive treatment of, for example, dried fruits.

THE REDUCTION OF LOSSES

In the past ten years, interest in the prevention and control of insect infestation in stored foodstuffs has grown rapidly and new methods, or improvements of existing methods, are being actively worked on. However, the utilization of available knowledge lags far behind; for that depends upon persuading everyone from farmers to marketing administrations that control is an economic advantage and, especially in under-developed countries, upon setting up organizations to distribute insecticides and instruct on their correct use. In suitable circumstances, simple treatments can be highly effective (19, 21, 22, 23, 25, 32) and very low in cost compared with the value of the product saved (22, 25, 31, 40).

Many accounts of general control measures are available (e.g., 41 to 48). Others deal with the special needs of individual countries (e.g., 49 to 57); the United States is particularly well served by the numerous bulletins and reports from the United States Department of Agriculture and from State Agricultural Experiment Stations. Moreover, many papers describe control of insects in particular types of storage (silos, mills, bakeries, rolling stock, etc.), in particular products, by particular methods, or with particular insecticides. Space does not permit their individual mention, but an exception must be made for Monro's excellent account of ship infestation (58).

To give a broad picture of modern methods for reducing losses and providing cleaner food, recent and current investigations will now be reviewed, pointing out, in passing, one or two of the more neglected lines of work.

Control by hygiene.—Surprisingly, the benefits of hygiene seem to have received no scientific assessment, although the need for good stacking, sweeping, vacuum cleaning, and efficient disposal of infested debris is being continually, and rightly, stressed in both general and special (59 to 62) publications. The measurement of insect populations by detailed sampling techniques, employed at intervals over two or three years, in cleaned and non-cleaned premises should yield interesting results.

It is also high time that engineers concerned with the design of silos, warehouses, factories, ships, freight cars, etc. consulted entomologists about modifications of designs to facilitate the prevention of infestation in stored or transported produce and the control of infestation if it should develop. Only two papers have been found dealing specifically with the design of machines and fittings to promote hygiene and to reduce insect harbourages (63, 64), although the need for modifications is stressed in others (e.g., 47, 58, 65, 66). A specialized contribution has been made by Cotton & Apt (67) who recommend a glass-wool packing between the inner and outer walls of boxcars.

Mechanical control.—Turning and screening of grain are often recommended as control measures, yet again there appears to be no published information giving scientific data on their value. Light traps are not used in the food industries, although common in tobacco stores as indicators of *Ephestia* and *Lasioderma* infestation; their value for control is negligible. Sticky banding of warehouse walls, formerly used in the United Kingdom to trap migrating caterpillars of *Ephestia* and *Plodia*, has now given way to more general use of pyrethrum sprays.

An invention of some 15 years standing which has gained steadily in popularity in factories and mills is the Entoleter (68, 69, 70) in which powdered products are flung centrifugally against steel pillars or the walls of the apparatus with sufficient force to kill all stages of insects.

A very recent suggestion is for the mechanical protection of clean goods, e.g., after fumigation, by storage under light-weight, insect-proof, cotton or plastic sheets bedded at floor level into a heavy layer of insecticidal dust (71). Under suitable circumstances this might be a cheap and simple method of preventing cross-infestation, but needs large-scale trial.

Control by sealed storage.—The need for long-term (3 to 4 years) storage of famine reserves is a pressing problem in some parts of the world. Workers in the Argentine started experimenting in 1941 with long-term storage of grain in pits in the ground (72), and pit storage is now receiving detailed experimental attention in other countries (73, 74, 75). Encouraging results are being obtained in preserving maize, etc. in an insect-free and edible condition for several years. Insect infestation in the grain is killed by the metabolic depletion of oxygen and, less effectively, by the accumulation of carbon dioxide in the pit (76). A similar principle is involved in the storage of grain in hermetically sealed bins above ground, these having potentialities especially for the storage of grain of moisture content between 16 and 20 per cent (73).

Control by nonradiant heat.—Nonradiant heat has obvious possibilities (47, 77) but is too expensive unless combined with drying, and driers have been developed almost entirely for cereals in temperate climates where infestation at the time of drying is negligible. Moreover, the rapid transfer of heat without damage to the product presents practical difficulties by requiring treatment of thin layers. Sterilization of flour mills by opening the buildings to sub-zero temperatures in winter is said to be successful in parts of North America (47).

Control by radiant energy.—(a) Nonionizing radiations: high-intensity ultrasonic waves are lethal to insects but are too expensive a method of energy transfer for practical control of stored product insects (78).

High-frequency electric fields (77, 79 to 84) and infrared heating (47, 85, 86) have given good results under laboratory conditions, but both suffer objections, when considered for practical use, of high cost, slow throughput, and a small difference between the temperature and exposure period required to kill the insects and those that may damage the product. Infra-

red also suffers from poor penetrative powers compared with high-frequency heating.

(b) Ionizing radiations: ultraviolet light (86, 87) and x-rays (87) are ineffective against stored product insects.

The use of gamma-radiation from certain radioisotopes is considered economically feasible, but much work clearly needs to be done before a safe and easily workable plant can be evolved (88, 89).

Lastly, recent investigation of the insecticidal properties of accelerated electrons suggests that they could be used effectively at low running cost, especially if the capital cost of the installation could be made reasonable (90, 91, 92).

Control by fumigation.—Fumigation has long been an important method of control of insects and mites in stored produce. Many general accounts of modern methods are available (e.g., 6, 43, 44, 55, 93), but there has been much recent research on and development of special techniques, mostly to obtain better distribution of the gas in the product by improved stirring, by forced circulation, or by the use of a vacuum. Major factors in the development of these new techniques have been, for example, proof of the great penetrative power of methyl bromide; the relative safety in use of the chlorinated and brominated aliphatic hydrocarbons; and the increased use of gas-concentration measurements at intervals of time in different parts of the product to investigate the behaviour of the fumigant and to adduce reasons for successes and failures in practice.

Advances have been made in methods for treating ships' holds (58, 94) silo bins (94, 95), hangar stores (6), farm bins (96, 97), boxcars (98), and empty sacks (99). A reinvestigation of the established techniques of vacuum fumigation has shown that neither sustained vacuum nor initial simultaneous admission of gas and air are so efficient as initial admission of gas and delayed release of the vacuum with air (99, 100).

British workers have given much attention to fumigation of commodities under gas-proof sheets, and the technique can now be adapted to cope with stacks from a few bags in a building to a huge pyramid of bagged groundnuts in the open (101, 102).

Other important research has been done on taint and residues in fumigated foodstuffs (103 to 106); on the treatment of elevator boots by local application of compounds of low vapour pressure such as hexachloropropene (107); and the relative resistance of the commoner species of stored product insects to various fumigants (101, 108). Ethyl bromide has also shown promise as a new fumigant suited to Indian conditions (109).

Control by contact insecticides.—The practical use of contact insecticides for the control of stored product insects has tended to outstrip properly contrived experimental work, especially with chemical dusts, sprays, and smokes. The result is to make it hard at times to draw the line between scientific contributions and pseudoscientific papers that are little more than advertisements.

(a) Inert mineral dusts: The protective value of inert mineral dusts was extensively investigated in several countries during the second world war. Their disadvantages under most conditions of use outweigh their advantages, but they are occasionally useful for application in dry localities to beans (19, 110) or grain (111, 112).

(b) Chemical dusts: Because of their low mammalian toxicity, dusts containing pyrethrins or pyrethrins synergized by piperonyl butoxide have been developed for mixing with grain to give marked protection against insect attack (20, 21, 24, 40, 113 to 117). Under some conditions, however, these dusts have proved costly or disappointing in their performance (25, 32, 118 to 121).

Many countries do not permit DDT or lindane dusts to be used directly on stored foodstuffs, although restrictions are slightly less severe on lindane (2). Attention has, therefore, been directed to tests under special circumstances where these dusts might be used without risk. For example, DDT dusts can control bruchid infestation in beans (110) and rice weevil in bagged maize (22), but many species of stored product insects are relatively resistant to this insecticide (122). Lindane dusts, too, can prevent bruchid attack in beans (22, 110) and rice weevil attack in crib-stored maize (21) and in bagged shelled maize (20, 22). Much experimental work is now proceeding, especially in British colonial territories, where these dusts are the only cheap, available insecticides applicable without special apparatus.

(c) Sprays: Certain mineral oils have been found highly specific in their insecticidal effects and can be used against suitable infestations by moths (123) and grain weevils (124). Pyrethrum sprays are now giving way to the cheaper and equally effective pyrethrum-piperonyl butoxide mixtures applied as oil solutions or emulsions (125, 126, 127).

DDT and lindane wettable powders and emulsions are well established for treatment of the walls, etc. of empty stowages against residual infestation (30, 121, 125, 128) and in dry climates can be applied instead of dusts to sacks containing grain (22), as the contamination will be less (129).

(d) Smokes: Smoke generators are a fairly recent addition to the methods of applying DDT and lindane. They are simple, portable, self-contained, and particularly useful for the treatment of residual infestation in thoroughly cleaned empty premises, e.g., farm granaries, warehouses, and ships' holds, as well as mills (130 to 133).

(e) Sack impregnation: Tests of sacks impregnated with pyrethrins or pyrethrins-piperonyl butoxide to protect the contained foodstuffs against infestation have shown this safe treatment likely to be rather costly for commercial jute sacks (134 to 136). Better and cheaper protection can be obtained with DDT or BHC impregnation but unfortunately at the risk of excessive contamination of some products (26, 28, 137 to 139).

(f) Package protection: The problem here is that of protecting packaged foods after leaving the factory, i.e., during transport and shelf storage. All the commonly used packaging materials can be penetrated by insects, but

some of the tests, all made under laboratory conditions, may have been too severe (140, 141, 142). The inability of the closures to exclude insects is clearly a major weakness of present package construction. Pyrethrum-piperonyl butoxide applications seem the most promising (143), although it is not yet clear how long they will remain effective when exposed to light under normal storage conditions.

CONCLUSION

There is already an imposing array of data about losses caused by insects to stored foodstuffs which must raise the question whether sufficient attention is yet being given to dealing with this source of food wastage and spoilage. There is also good evidence that the small band of workers in stored product entomology have in recent years built up a background of experience and knowledge which, if more widely applied, could effect immediate and extensive saving of foodstuffs. In hot climates, in particular, the value of these savings would be greatly in excess of the cost of the control measures. Unfortunately, the application of such measures lags sadly behind the advance of knowledge in all but the most highly developed countries, and even in those, is not always so widespread or up-to-date as it might be.

Nevertheless, there remain many problems to be solved. For example, more needs to be known about the specificity of the insecticides in common use; many new insecticides await evaluation under the special conditions of use on or near stored foodstuffs (and on other stored products which have not been considered in this review); more opportunity is wanted for field experiments and demonstrations to be done in the regions where control is needed; and more information is urgently required on the chronic toxicity of insecticides and on the levels of contamination of foodstuffs that may be expected in normal use. More support for such projects can be expected as the realization grows of the extent of preventable losses, and a plea is entered here for the deliberate planning of surveys and experiments to yield information in a form that will permit comparison and summation of losses under widely differing conditions of infestation. The fact that the front of knowledge in stored product entomology is steadily advancing must not lead to complacency. The insects are still firmly entrenched in many of their strongholds and can still effect sorties and surprises, as witness the recent appearance in California and the neighbouring States of *Trogoderma granarium* Everts, an insect for which no really effective control exists beyond difficult, extensive, and costly fumigations (144).

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APPARATUS FOR APPLICATION OF INSECTICIDES¹

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EARLY HISTORY OF APPLYING EQUIPMENT

Recent developments in new methods and equipment for the application of insecticides and fungicides have stimulated a great deal of interest in this field. At first glance it would appear that most of the developmental work has been done in the last 10 years. Actually this recent surge in new machines is the result of at least 75 years of research into the problems involved in applying pesticides to plants. Many of the basic ideas now utilized in our most modern sprayers and applying equipment were expressed and tested 25, 50, or even 75 years ago. Further development of these ideas at those early dates was curtailed by the lack of adequate insecticides or fungicides, the need for light, portable power units, and insufficient engineering know-how. During and since World War II economic pressure, the need for more food, and the lack of man power have made it imperative that we produce more per acre and more per man hour. To do this insects and diseases had to be controlled as effectively and efficiently as possible. Early in the 1940's the chemist, who had been trying to synthesize new insecticides and fungicides for years, developed new and promising materials. The entomologist and plant pathologist found these chemicals to be very effective, and because they had acquired a great deal of knowledge of the ecology and biology of the insects and plant disease organisms they were able to use these materials in new and effective ways. And finally the engineer was called upon to utilize his knowledge and experience gained largely in other fields, to design and build machines to apply these new chemicals in the most effective and efficient manner.

The development of machines for the application of plant pesticides follows hand in hand with the development of chemicals for the control of insects and diseases.

Excellent historical accounts of the development of insecticides and fungicides as well as the parallel development of machines for applying these materials are given in several publications: *The Spraying of Plants* by Lodeman, 1896 (40) discusses the problem of spraying plants from the early eighteenth century to the late nineteenth century, giving particular attention to the development of materials and equipment between 1860 and 1896. It was during this period that the first real advances were made in plant pest control through the use of chemicals. As indicated by the title, *The Advance of the Fungi* by Large, 1940 (39) is primarily a historic account of man's

¹ The survey of the literature pertaining to this review was completed in January, 1955.

quest of a method to control plant pathogens, but incidentally discusses the development of insecticides and early applying equipment. *Insecticides, Fungicides and Appliances* by Anderson & Roth, 1923 (2) is a laboratory manual for studying insecticides, fungicides, and applying equipment. It is valuable as a reference book for studying the status of plant pest control materials and equipment during the early 1920's. *Spraying, Dusting and Fumigation of Plants* by Hough & Mason, 1951 (34) covers nearly all phases of plant pest control from ancient times to the present. All of these books are well illustrated with pictures of various types of equipment. They also contain typical materials and formulations of times past with the recommendations for their use in the available machines. Since the above accounts are easily available and complete, the present discussion will allude to the early history of equipment only insofar as it helps us to understand the present status and future prospects.

Prior to 1868 there was little or no knowledge regarding chemicals for the control of plant pests and, therefore, no need for applying equipment. Famines caused by insect outbreaks and epiphytotics of plant diseases had come and gone but no effective means had been designed to prevent or control these catastrophes. In the early 1850's the Colorado potato beetle left its home near the foot of the Rockies and began moving eastward on the intermittent potato fields established by the westward migration of the pioneers. As they spread the beetles destroyed entire fields of potatoes making it impossible to produce a satisfactory crop. Thus, this insect threatening one of the staple food crops of the entire country presented a very important control problem. Someone whose name seems to be lost in history tried a dust of Paris green diluted with an inert material such as flour, lime, or even wood dust. Lime finally became the standard diluent when it was found that this formulation caused less burning to the plants. The applications were made by means of a box with a wire screen bottom or a loosely woven cloth bag, either one of which was filled with the mixture, held over the plants and shaken so that some of the dust fell on the leaves. This crude arrangement was probably the first widely used type of applying equipment used in the United States.

In the meantime the people of France were faced with a problem that threatened to wipe out the wine industry of the country. In 1878 the grape vine mildew had first been observed by Millardet and Planchion [Large (39)]. By 1882 it had spread to nearly all the grape growing areas of France and was beginning to cause severe losses in parts of Germany and Italy. It was in this same year that Millardet made his historic observations on the effectiveness of the copper sulfate-lime combination which eventually came to be known as Bordeaux mixture. Unlike the Paris green dust, this new material was a liquid and so presented a new problem in application on thousands of acres of vineyard. Lodeman (40) describes and illustrates the heath whisk, a crude whisk broom made of heath straw which was used for

the first applications of Bordeaux. The brush was dipped into the thick copper sulfate-lime mixture and then swung at the vines, thus splattering the foliage with the material that flew from the brush. He also describes an improved brush (40, p. 182),

The liquid, which was carried in a tank on the back of the operator, entered the hollow handle through a tube connected with the bottom of the tank. The fluid then ran along the fibers of the broom and was thrown from the extremities.—A stopcock was inserted in the handle so that the liquid could be shut off when desired.

The coverage produced by this device was very uneven and inadequate by present standards, but to quote Lodeman again (40, p. 182), "For many plants it is surely much better to use a device of this character than it is to make no application . . ."

Once the materials for plant pest control were available machines for their application began to appear. Large (39, p. 229) pictures and describes several machines developed in Europe.

One of the first contrivances, invented by M. Armand Casenave of la Reola, had a revolving brush, operated through a chain of gear-wheels and fed with mixture from a portable copper tank to which it was attached. On turning the handle, a stirrer went round in the tank, the brush went round in its trough, and a scraper pressed against the bristles causing them to project the mixture in fine style.

Thus, the spinning brush used in the early work on aircraft application was used long before the airplane was born. Of particular interest in the light of the recent trend toward air blast and concentrate sprays is "Le Projecteur Audebert" pictured by Large (39). In the case of this machine the operator carries a large pair of domestic bellows in his hands and a small tank on his back. The tank is connected to the discharge end of the bellows with a small rubber tube. The liquid trickles down the tube and is atomized onto the plants by the air from the bellows. Probably the first mist concentrate machine.

When one reads the formulations used in the early years of plant spraying there is no doubt but what concentrates were being used as measured by present day standards. For instance, Millardet's formula for Bordeaux mixture as translated and quoted by Lodeman (40, p. 27) from the *Annales de la Societe d'Ag. de la Gironde*, 1885, was as follows, "In 100 liters of water dissolve 8 kilos of commercial sulphate of copper. In another vessel make a milk of lime by slaking 15 kilos of quicklime in 30 liters of water. This is then added to the copper sulphate solution, causing a bluish precipitate." Translated into United States measures this approximates 18 pounds of copper and 33 pounds of lime in 34 gallons of water or equivalent to a 54—99—100 Bordeaux mixture. By 1887 Millardet had reduced his formula to 3 kilos of copper sulphate and 1 kilo of lime in 100 liters of water.

From 1870 to 1890 many new sprayers and nozzles were developed and

sold for treating plants. Foremost among these were the various types of knapsack sprayers and hand operated force pumps. Hough & Mason (34) point out that John Bean developed the first force pump sprayer at Los Gatos, California in 1883. According to Lodeman (40) the Nixon Nozzle and Machine Co. of Dayton, Ohio was the first to manufacture a geared spraying machine. This sprayer called the "Field and Orchard Machine" was offered for trial in 1887 and was first sold in 1888. It was a traction type machine, the pump being operated by a chain drive from the wheels. The pressure was controlled by a simple poppet valve which returned the excess liquid directly to the tank. Machines similar to this design continued to be used for spraying in some areas until the 1930's. The first steam powered sprayer made by Stephen Hoyt in New Canaan, Connecticut, was first used in 1894. This machine was unique in design in that the piston rod from the steam cylinder was connected directly to the piston of the pump. The liquid pressure produced by the pump was controlled by the steam pressure. Lodeman (40) quotes a letter from Mr. Hoyt which said that 100 pounds per square inch steam pressure produced a pump pressure of 125 to 150 pounds per square inch. Simultaneously with the appearance of the steam powered sprayer in Connecticut a gas engine sprayer was built by the Union Gas Engine Company in San Francisco. This machine was capable of operating at 200 pounds pressure. However, Hough & Mason (34) report that it was about 1900 before the first complete gasoline powered sprayers appeared on the market.

Thus, in 1900 only two major developments were lacking for the production of the high pressure sprayer as it is known today. In 1911 the John Bean Company invented and manufactured the first true pressure regulator and in 1914 the Friend Sprayer Company introduced the adjustable spray gun. From then until the middle 1930's, with one notable exception, equipment for the application of liquid fungicides and insecticides advanced steadily toward larger pumps, larger tanks, higher pressure, and higher rates of application. The exception was the appearance in 1925 of the Liqui Duster manufactured by the Rex Company of Rochester, New York and later purchased by the Niagara Sprayer and Chemical Company. Equipment for applying dust in an air stream had been progressing steadily, paralleling the advances in spraying machines. Hand puff dusters and rotary dusters soon took the place of the cloth sack and screen bottomed box; to be followed by by traction dusters and finally by those powered with gas engines. However, the Rex Liqui Duster anticipated by several years the application of liquid in an air blast. The liquid nozzle was mounted in the center and at the end of a flexible air tube. Standard dilute concentrations of materials were used and injected into the air stream. The air aided in the breakup of material and helped carry it to the trees. Because of the low volume and velocity of the air blast, some difficulty was experienced in getting coverage with this machine so that it fell out of favor in competition with the greatly improved high pressure sprayers that came onto the market at about the same time.

Between 1920 and 1950 so many new methods and equipment for applying insecticides and fungicides were developed that it would be confusing to discuss them all in chronological sequence at the same time. Therefore, an attempt will be made to point out the highlights in the development of several types of applying equipment with special emphasis on the status of each at the present time. This presentation will be largely limited to a discussion of equipment for treating trees and row crops exclusive of aerosols and aircraft applications. Aerosols are so limited in their use in these fields that they will be omitted. Aerial spraying is so broad in its application and some of its problems are so unique that it might be better covered in a separate article.

Dilute or high pressure spraying equipment has changed very little in the past 20 years. The use of larger tanks and higher capacity pumps have made it possible to develop spray masts and spray booms for the most efficient coverage of orchard and row crops. But these improvements have not materially cut down on the time for covering a given area, the amount of water to be hauled, nor the material required. It is true that dilute spraying is still being done extensively under certain conditions. As pointed out by Von Oppenfeld *et al.* (50), where a grower does not operate a large enough acreage to justify an investment in new types of equipment, dilute spraying is probably the most economic and effective method of application. Or, when is necessary to accurately control the distribution and amount of deposit on the fruit or foliage as in the case of fruit thinning sprays, applications for the prevention of harvest drop of apples, or for the foliar application of fertilizers, dilute applications seem to do the best job. However, because of the amount of labor and time required as well as the heavy weight of the equipment and the large amount of water needed in dilute applications growers have gone over to other methods of application wherever possible.

AIR BLAST SPRAYING

Dilute sprayers.—The first successful large air blast dilute sprayer was invented by G. W. Daugherty and made its first appearance in Florida citrus groves in 1937. This machine originally designed as a spray duster has since been modified into the now familiar Speed Sprayer. The wet dust feature was dropped in the late 1930's, and the four bladed aircraft propeller was replaced by an axial flow fan in the early 1940's [Daugherty (24)]. This machine in principle and in type of air and liquid discharge has been the basic pattern for most of the dilute air blast equipment sold today.

The Speed Sprayer was so successful in its control of pests of citrus that it was rapidly introduced to fruit growing areas northward to New York and Michigan and westward to California and Washington so that by 1945 it was found in all the major fruit growing areas of the United States. Since that time all the major spray equipment manufacturers have put one or more models of similar machines on the market. These machines are similar in

that they all deliver large volumes of air, from 10 to more than 40 thousand cubic feet of air per min. at average velocities of 85 to 150 miles per hr. In addition to the volume and velocity of the air, they vary in the nozzle type, the nozzle arrangement, and the liquid pressure which varies from 60 to 600 psi. Such wide discrepancies in the specifications for machines supposedly designed for the same or similar purpose require an explanation. Some of these sprayers, particularly those with the larger air blasts depend on the air to break up and carry the material through the trees and, therefore, can use lower liquid pressure. Those using high pressure usually produce less air blast depending largely on the pressure of the liquid to break up and carry the material to the trees. In this case the air blast aids in the distribution of the material by agitating the leaves on the trees, thus giving greater penetration and better coverage than otherwise would have been achieved. Although there has been considerable debate as to the relative merits of these two systems, the fact remains that fruit growers seem to be getting satisfactory results with both when the machine in question is adequate for the problem and when reasonable care is taken in making the applications. Because of the low point of delivery characteristic of this type of machine, there is a tendency to over spray the lower limbs and under spray the top center of the trees. This problem is particularly serious in large trees with heavy foliage and when concentrate applications are made.

Concentrate sprayers.—The idea of using an air stream to carry a concentrated finely divided suspension or solution of toxicant is not new. In fact, as pointed out earlier some of the rather crude sprayers designed as early as 1885 used this principle. However, Potts must be credited with initiating the modern trend toward the use of air to carry finely divided mists of highly concentrated insecticides [Potts & Friend (54)]. Although the equipment he used was preliminary in design and the air blast and velocity was inadequate, as measured by present standards, he did get coverage up to a height of 35 to 40 feet on shade and fruit trees. In 1933 Parker (51) reported on work done in 1931 with the "vapo duster," a machine which used an air stream to carry finely atomized oil for the control of the grape leaf hopper. In 1934 French (25) published a description of an air blast machine for applying finely atomized oil. He recognized the importance of droplet size and the effect of liquid pressure and air velocity on the size of the droplets produced.

In 1946 Potts & Friend (54) described and discussed mist blowers for applying concentrate sprays for the control of pests on shade trees and fruit trees as well as for the control of mosquitoes. They emphasized nozzle design and droplet size as well as volume and velocity of the air stream. By 1947 mist concentrate spraying of shade trees was rapidly becoming a standard practice. At least three commercial machines were in production at that time. The Buffalo Turbine Agricultural Equipment Company released its first commercial model in 1945; the Lawrence Aero-Mist Company began manufacturing their shade tree machine in late 1946; and some operators

were using John Bean's Model 7 and 17 Mist Dusters for treating shade trees with mist concentrates. At the Mist Blower Conference held at the Connecticut Agricultural Experiment Station in March, 1948 seven commercial mist concentrate machines specifically designed for treating shade trees were demonstrated.

Potts & Friend (54) reported on work conducted in co-operation with the Division of Fruit Insect Investigations of the Bureau of Entomology and Plant Quarantine at their Poughkeepsie, New York Laboratory in 1945. In this case a solution of 16 ounces of nicotine alkaloid per gallon of kerosene applied at the rate of 4 to 8 ounces per tree gave 90 to 96 per cent control of adult pear psylla. In 1948 Brann *et al.* (11) reported on applications of dormant oil made to apple trees with a Niagara duster modified to apply mist concentrates. They presented data showing the effect of concentration and rate of application on the amount of oil deposited and the effect of oil deposit on European red mite control.

Semiconcentrate sprayers.—Up to this point in this discussion the air blast machines mentioned have been using either dilute concentrations of toxicants (the same concentrations as those recommended for high pressure spraying) or concentrations 8 to 25 times as high as those recommended for high pressure spraying. The former might be designated as dilute air blast sprayers and the latter true concentrate machines. With the success of the high concentrate machines it was a natural step to try higher concentrations in the dilute air blast sprayers, thus, saving on the amount of water and time needed for making an application. Originally dilute spraying used large volumes of water to impart enough energy to the spray stream to carry the toxicant to the foliage to be treated. Even distribution of the toxicant was obtained by thoroughly soaking all parts of the plant or tree, and the rate of deposit depended directly on the concentration of the material in the tank. If the plants were not thoroughly wetted less deposit was obtained. On the other hand if the plants were over sprayed the excess ran off. In air blast spraying the droplets of material are carried largely by the air stream so that less liquid may be used.

Spraying operations in which the concentration of the material is two to four times that used in dilute spraying and in which one-half to one-fourth of the amount of liquid is applied may arbitrarily be called semiconcentrate. Borden (6, 7) reports on the use of semiconcentrate and concentrate sprays on deciduous fruit in California and emphasized the necessity of a large volume of air to obtain adequate coverage of the fruit and foliage. Burrell (18) published a description of the specific changes that should be made to convert a Speed Sprayer for use as a semiconcentrate machine at 2 to 4 times concentration. He emphasized the proper arrangement of the right sized nozzles for adequate distribution of the material in the trees.

Since 1948 there has been a tremendous increase in the use of air blast machines for treating fruit, shade, and forest trees as well as for control of

many other insect and disease problems. Reasons for this trend can be found in several publications. Miller *et al.* (47) reported that a dilute air blast machine using one man covered an orchard 2 to 2½ times as fast as hand operated spray guns operating from a high pressure sprayer using three men and required about the same time as a mist sprayer using two men. In amount of material used the mist sprayer, air blast machine, and hand gun sprayer used 27.4, 22.1, and 17.6 gallons per tree respectively. The difference in amount of material used between the air blast machine and the hand operated gun outfit would not pay the cost of the extra two men. Von Oppenfeld *et al.* (50) reporting on a two year survey of spraying operations on more than 100 fruit farms in New York State found that on the average the farm manager had a greater effect on the cost of production and quality of fruit than the spraying equipment or spray program, but did present data showing that air blast machines required only $\frac{1}{3}$ to $\frac{2}{3}$ as much time to cover apple trees as did other types of equipment. Also Borden (10) discussing deciduous fruit spraying in California pointed out that semiconcentrate and concentrate methods of application saved up to 70 per cent in labor and 20 per cent in materials. Thus with the cost of material and labor rising continually the growers had to seek the most economical method of application.

It is interesting to note that the trend has been toward semiconcentrate rather than concentrate equipment. This can perhaps be best explained on the basis of the desire of the average grower to change slowly. Also from the manufacturing point of view it was simpler and less costly to encourage growers to buy dilute air blast machines that could also be used for semiconcentrate applications than it was to produce true concentrate machines and educate growers in their use. Since most of the machines used for semiconcentrate applications were designed for dilute spraying, growers using them for semiconcentrates can go back to dilute spraying if they wish by simply changing the size and arrangement of the nozzles. As pointed out by LaPlante (38, p. 6),

Machines designed to operate primarily for higher concentrate spraying are generally engineered to operate at a 5X to 10X concentration although they have been used as high as 16X under special experimental conditions. It is usually not practical to go below 5X with such machines because of limited pump capacity.

He might also have added that the lower volume of air (usually $\frac{1}{3}$ to $\frac{1}{2}$ that of the dilute air blast machines) which is adequate for carrying say 5 gallons per min. of an 8X concentrate spray is not sufficient to carry the 50 gallons per min. which would be necessary for a dilute application. Thus a grower with a true concentrate machine is usually limited to making applications of relatively high concentrations.

DUSTING EQUIPMENT

A discussion on applying equipment would not be complete without a word on dusting. Power dusters came into production at the turn of the cen-

tury and have been constantly improved to the present time. Dusters have had the advantage over dilute sprayers in that the machines cost less and that they cover ground faster. They have certain disadvantages in that they tend to deposit less than sprayers and the deposit weathers faster than those applied in liquid. Also insecticides formulated for dust application tend to cost more per unit of toxicant than the same material formulated for spray application. For example, Hamilton (30) presented data comparing nine dust treatments and six spray treatments for the control of codling moth in which approximately 25 per cent more total toxicant was applied during the season in the dust as in the sprayed treatment. At harvest the dusted fruit had more codling moth injury and less residue per unit of toxicant applied than that which was sprayed. Another weakness in dusting equipment has been the difficulty of metering the dust out at an even rate. Glaves (28) developed an even feed dust hopper which operates very effectively but has been used very little in commercially produced equipment. This may have been a result of the fact that since that time the swing has been toward liquid air blast equipment. Dusting is still used on many crops as the principal method of application and on others for rapid application in case of emergency. However, the improvements in air blast sprayers have so cut down on the time, labor, and materials for such applications that they are generally preferred over dusters wherever possible and practical.

WET OR SPRAY DUSTERS

The process of wet or spray dusting came into being in an attempt to utilize the best features of both spraying and dusting. In this operation the toxicant was carried in dust form, but as it left the duster outlet a small amount of water was sprayed into the dust stream which partially wet the dust and also laid down an artificial dew on the foliage. This procedure resulted in heavier initial deposits and a longer retention of the material. Hamilton & Mack (31), Barnes (4), and Pratt (58) all pointed out the advantages of spray dusting and presented data to show that it gave excellent control of insects and diseases equal to or better than high pressure spraying in 1946. Pratt (59) described the new Cornell Spray Duster pointing out the advantages of the elongated slot type of outlet and presented data to show excellent control of apple scab and cherry leaf spot. In 1947 Pratt (60) compared the results obtained when this same machine was used as a wet duster and as a mist concentrate machine. He summarized the experiment by stating, "In these experiments results obtained with mist sprays were as good or in some cases better than with spray dusting or conventional sprays applied with a gun." Borden (8) stated that some manufacturers had a duster attachment on their air blast equipment, but he does not give any indication whether or not these machines were used as wet dusters. From observations as well as from the lack of reports in current literature it is apparent that wet dusting has given way to concentrate and semiconcentrate applications.

EQUIPMENT FOR ROW CROPS

The development of new equipment for treating row crops has in general followed the same trend as that followed in the case of fruit. Namely a search for machines that would do the job with less liquid, less man power, and less toxicant in less time. Brann & Gunkel (14) set up a list of requirements for a low volume sprayer in which they stress economy of weight, liquid, toxicant, and cost. They also emphasize the necessity for adequate coverage for disease as well as insect control. Low volume spraying of row crops has taken two approaches: One is air blast machines, concentrate or semiconcentrate, and the other is low gallonage sprayers. In low gallonage spraying usually only $\frac{1}{2}$ to $\frac{1}{3}$ the amount of water is used as would be needed for conventional high pressure or dilute spraying, the concentration of toxicant in the liquid being increased in proportion as the gallons per acre is reduced. Low pressure 50 to 80 psi is used while distribution is obtained by using small nozzles that break the spray into a very fine mist. Drops may or may not be used depending on the crop and the pest to be controlled. Low gallonage spraying for insect and disease control is a direct development from the low pressure weed control sprayers developed during the late 1940's. In 1949 Medler & Chamberlain (46) who had shown an increase in seed yields from red and ladino clover when insecticides were applied with a weed sprayer at about 30 psi and 100 gallons per acre in 1947, reported very good control of forage insects applying from 15 to 30 gallons per acre at 30 to 40 pounds pressure. In 1952 Bonde *et al.* (5) in Maine comparing 100 to 150 gallons of liquid per acre with the high pressure sprayer and 25 to 35 gallons per acre with the low gallonage machine in 1950 and 1951 reported excellent aphid control in both years. Referring to the low gallonage sprayer they said

This machine gave complete control of late blight in 1950 under favorable conditions for spread of the disease. However, in 1951 when conditions for late blight spread were extremely favorable, the high pressure spray machine controlled the disease somewhat better than did the low pressure outfit.

In their report for the four years 1950 to 1953, Peikert & Bonde (53) show the low gallonage treatments to be nearly as effective as the high gallonage applications in control of late blight and early blight of potatoes. The machines were equal in aphid control and both gave satisfactory control of flea beetles. After going into considerable detail on the engineering and entomological aspects of low gallonage spraying Hervey & Gunkel (32) conclude that "The low volume method of insecticide application using a weed control type sprayer is a practical means of insect control for certain vegetable crops." They were using 15 to 20 gallons of spray per acre applied at 40 to 80 psi. Brann & Palm (16) point out the advantages and disadvantages of low gallonage spraying. It is necessary to adjust the boom for each crop, and the boom must be lifted for turning at each run across the field. Also the adjustment of the nozzles to assure coverage of each row is critical. Because of

their abrasive effect on the pump and nozzles and also their tendency to clog the fine nozzles, wettable powders cannot be handled satisfactorily. This limits the formulations to solutions or emulsions, which excludes the use of some insecticides and many fungicides.

In spite of these limitations the advantages of low gallonage far outweigh the disadvantages. Satisfactory insect control has been reported by many workers on a wide variety of crops using as little as 8 gallons per acre on cotton to 20 to 40 gallons per acre on beans and cabbage. This type of application has also shown a great deal of promise in the control of insects of turf [Matthysse (45)] and forage [Marshall *et al.* (41)] insects. It offers a great saving in the cost of equipment and in the time and labor required. The coverage obtained has been sufficient to give adequate control of a number of insect pests, but more work is needed before a definite statement on disease control can be made.

The development of air blast equipment for row crops presents unique problems in the deposition and distribution of insecticides and fungicides. As pointed out by Brann & Palm (16),

Attempts to pipe the air and liquid out to individual rows has not proven satisfactory for most uses. Such designs must have cumbersome booms and so little liquid must be delivered through each nozzle that clogging becomes a serious problem. Most models now being tested or offered for sale cover several rows with each nozzle.

Bronson & Rust (17) discuss their work with a mist concentrate machine for 1947, 1948, and 1949. In 1947 and 1948 the work was done with an improvised mist blower fitted with four air outlets which covered a swath 12 ft. wide. In 1949 they had a greatly improved machine which delivered 3500 cu. ft. per min. of air at a velocity of 5500 ft. per min. Four fishtails with 24 by 2.25 in. outlets connected to a tubular boom distributed the air over a 20 ft. swath. They reported excellent control on a number of insect pests including the potato flea beetle, the potato leafhopper, three species of cabbage caterpillars, and onion thrips using 10 to 12 gallons of liquid per acre. Potts and co-workers (57) worked with a machine manufactured by the Homelite Company delivering 1500 cu. ft. per min. of air and compared a fishtail with an arrangement of three round outlets set in a plane and diverging about 15° from each other. Both outlets were mounted about 5 ft. above the ground and depressed 15 degrees toward the ground. He found that the fishtail gave an effective swath width of 10 ft. while the same amount of air through the three round outlets gave an effective swath width of 15 ft. Using this machine he obtained excellent control of the Mexican bean beetle on beans, the potato aphids on tomatoes, and red mites on young nursery stock at six gallons of liquid per acre. Brann & Gunkel (15) described a rather radical mist concentrate machine for row crops patterned after the Mist-O-Matic orchard sprayer developed earlier. The distribution system in this machine consists of four round outlets mounted on a turret

rotating in a horizontal plane. The outlets are depressed 16 degrees from the horizontal. The fan delivers 4,000 cu. ft. per min. of air which is discharged through the outlets during the forward 180 degrees of rotation. The liquid is injected into the air stream during the forward 120 degrees of rotation. Thus, as the machine moves across the field it lays down a series of cycloidal swaths 2 to 3 ft. apart. Although the machine appears to lay down a swath 20 to 24 ft. on each side of the line of travel, data is presented to show that the effective swath width is between 20 and 24 ft. When a complete overlap of the swaths is obtained by crossing the field at intervals of 20 to 24 ft. an even deposit was laid down across the swath as well as uniform coverage on both sides of the leaves. This machine appeared to be so promising in the control of a number of pests of smaller row crops at 5 to 10 or 20 gallons per acre that a larger one (Fig. 8) was built in 1954 with an effective swath width of 35 ft. So far this machine has given excellent control of cabbage aphid on broccoli.

Most manufacturers of equipment have not been inclined to produce true concentrate machines for use on row crops. The equipment now offered for sale is, for the most part, adapted or developed from air blast sprayers designed for fruit trees. The air blast from these machines is usually rated from 20,000 to 40,000 cu. ft. per min. and is directed at right angles to the direction of travel. In some cases the air blast is delivered from both sides of the machine, sometimes from only one in an attempt to cover swaths of 60 to 80 ft. wide. Recommended rates of application vary from 50 to 100 gallons per acre. The rows over which the machine passes is usually treated with a low gallonage boom. Since these machines have only recently come on the market and so few carefully controlled experiments have been run with them, it is difficult to evaluate their effectiveness in insect and disease control under severe conditions. Observations and limited experimentation in New York State have indicated that where the air blast is large enough and where the swath is kept down to 40 or 50 ft. insect control can be obtained. In a single experiment on tomatoes under conditions of moderate infection there was no difference in the control of anthracnose and early blight between a high pressure machine applying 200 gallons per acre, an air blast machine (Fig. 12) with a 48 ft. swath applying 50 and 100 gallons per acre, and the experimental concentrate machine at 20 gallons per acre. In this case the air blast machine delivered from both sides, and the swaths were completely overlapped.

Problems in air blast spraying.—In dilute spraying the foliage of the treated plants is wetted to the point of run-off for most effective distribution and deposition of the toxicant, and the amount of deposit is controlled by the concentration of the toxicant in the spray mixture. If too little liquid is applied the deposit may be insufficient for adequate control. However, if too much is applied the excess runs off. In concentrate or semiconcentrate spraying the foliage is never completely wetted. Thus, there is a tendency

for the deposit to increase as the rate of application increases to the point of run-off. Brann *et al.* (11) show this effect on deposits of dormant oils when the rate of application or the concentration of oil is increased. Brann (12) showed a similar effect on deposit as well as in codling moth control when the rate of application of toxicants to foliage was increased. Many workers have pointed out the need for carefully controlling the concentration as well as the rate of application [e.g., Borden (10); LaPlante (38)]. Brann (13) and others have pointed out that the amount of deposit obtained with concentrate sprayers depends on three factors: the concentration of material in the tank, the rate of liquid discharge, and the rate of travel. Borden (10) emphasizes the rate of travel among other factors, and Gunkel (29) describes a low reading speedometer available for mounting on tractors or spraying equipment. Borden (8), Burrell (19), and Asquith (3) emphasize the necessity for a large air blast while Ingerson & Irons (35) point out the necessity for the proper balance between volume and velocity of the air as well as the proper size and arrangement of nozzles. They further state that the "air moving capacity of the machine should be sufficient to agitate all the air within the tree and displace much of it." The volume and velocity of air needed to do a given job seems to be a much debated question. Brann (12) points out that air delivered through round outlets maintains its velocity over a greater distance than the same amount of air delivered through outlets of other shapes. Potts *et al.* (57) obtained 50 per cent greater swath width with three round outlets than he did with a fishtail type. Commercial machines on the market vary tremendously in air blast as well as in air discharge mechanisms. A review of the literature on this problem indicates that the volume and velocity needed depend for one thing on the concentration used as well as the type of air delivery. The air blast from a dilute air blast machine spraying from both sides might be required to carry 50 to 75 gallons or in excess of 400 pounds of liquid per min. whereas a concentrate machine operating from one side at 8X or 10X may be only carrying 4 or 5 gallons per min.

An air blast is inherently a poor carrier for liquid droplets. It loses its velocity quickly, and the droplets tend to settle out. As Potts (55) points out, "Other things being equal the distance over which blowers can drive a cloud of finely atomized spray mist is directly related to particle size." He found that in the same air stream droplets of 60 to 80 μ in diameter were carried 150 ft. while droplets of 300 to 400 μ in diameter were driven only 20 to 40 ft. Akesson (1) advises that an average droplet size of 75 to 100 μ may be best for blower sprayers. He also points out that the number of droplets in any given volume of liquid varies as the cube of their diameters and that theoretically the smaller the droplet that can be deposited the better the coverage. But he also cautions that very small droplets fail to deposit and increase the drift problem. Marshall (43) says, "It has been calculated that greatest efficiency in spraying comes with droplets ranging from 30 to 80

microns in diameter"; but that "droplets above 100 microns in diameter constitute about 50 per cent of the total quantity of liquid. One of the most interesting problems in concentrate spraying is the reduction of these large spray droplets to a more efficient size." Thus, the droplet size problem in air blast spraying is very real and becomes more and more important as the concentration is increased and the rate of application is decreased.

Coverage.—Complete coverage of the plants and even distribution of the material are emphasized by many workers. Data are presented by Brann (13) to show the differences in coverage obtained from top to bottom of trees as the pattern of delivery of a Mist-O-Matic sprayer was varied. He found that it was necessary to aim five times as much liquid at the top of the trees as at the bottom to get an even deposit of insecticide. Marshall (43) mentions that there is a tendency for concentrate machines to over spray the lower branches of the trees and shows a picture of the scaffold device he used to make preliminary tests on the distribution of deposit laid down by various machines. In his recommendation to growers LaPlante (38) urges them to direct several times as much material toward the tops of the trees as at the lower limb, and Klingbeil & Mitchell (37) illustrate through the use of leaf prints the differences in deposit on leaves picked from the top, inside, and bottom of apple trees sprayed at 2X and 4X concentration.

In spite of all the problems in air blast, semiconcentrate, or concentrate spraying this method of application is rapidly being accepted by growers in many fruit growing areas. It has been estimated that 80 per cent of the fruit in New York is now sprayed by air blast at some degree of concentration. Marshall (43) stated that about 90 per cent of orchards in British Columbia were protected by automatic concentrate sprayers in 1953. While Mitchell (48) says, "It seems safe to state that probably 10 percent of the Michigan fruit growers representing 15 percent of commercial production will be using concentrate spraying in 1953." Figures for other areas have not been encountered, but indications are that the trend is toward air blast and concentrate equipment. Many foreign countries are interested in and are using these new methods of application.

Articles by Ripper (61), Stapley (62, 63), Clark (20), and Courshee (21, 22) indicate the interest of the growers and the extensive research being done on low gallonage and concentrate spraying in England.

Development and acceptance of air blast or concentrate methods for row crops has been slower. Although many of the problems met in this field are similar to those found in orchard spraying there are some that are unique and need a great deal more study. The fact that some growers are using air blast equipment on row crops, will probably stimulate more work than is now being done. The work underway at present seems to indicate that many insect control problems can be handled by this method, but much more data are needed on disease control before it will become a general practice with growers of row crops.

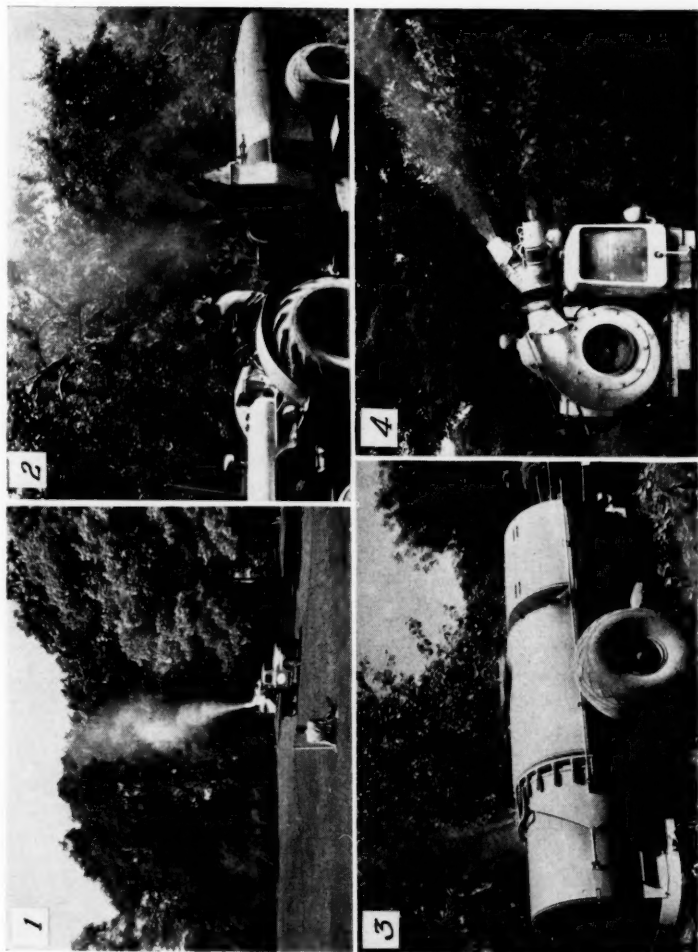


FIG. 1. Mist concentrate machine for treating shade trees.

FIG. 3. Speed sprayer applying semiconcentrate at 4X concentration.

FIG. 2. Hardie orchard mist sprayer with long-slot outlet, 6X to 8X concentration.

FIG. 4. Mist-O-Matic type with rotating head designed for 8X to 12X concentration.

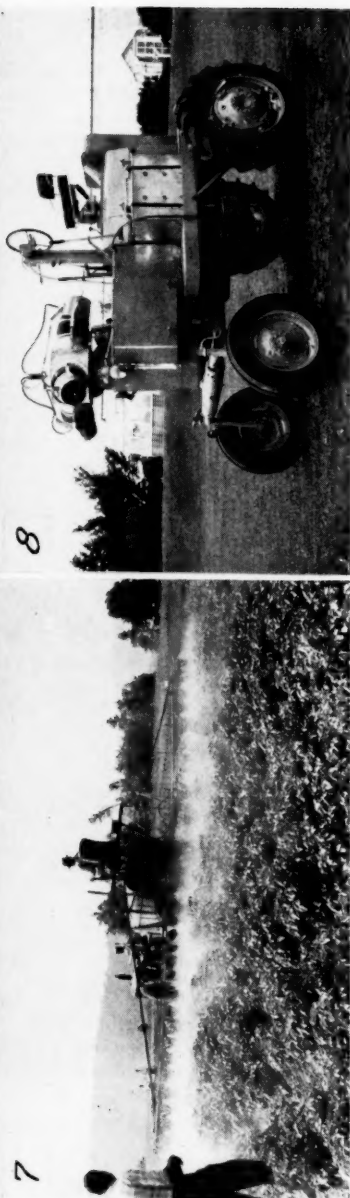
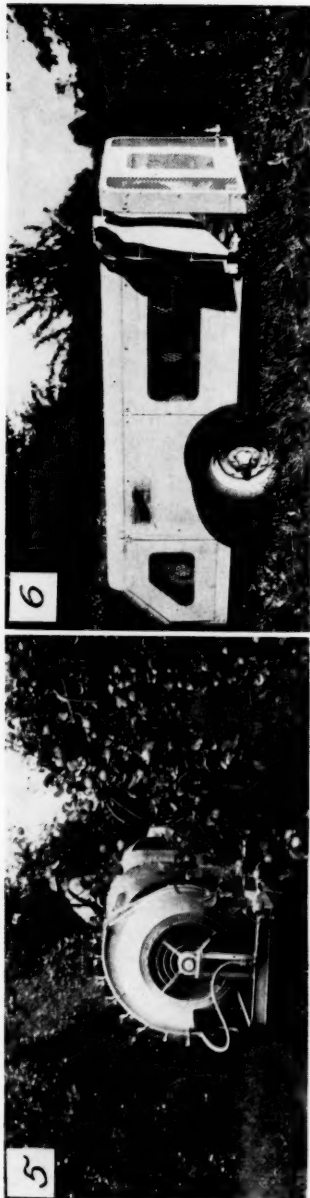


FIG. 5. Myers concentrate sprayer with oscillating outlets applying semiconcentrate spray.

FIG. 7. High gallonage-high pressure sprayer, 100 to 300 gallons per acre.



FIG. 6. Iron Age concentrate machine with key-hole slot outlet.

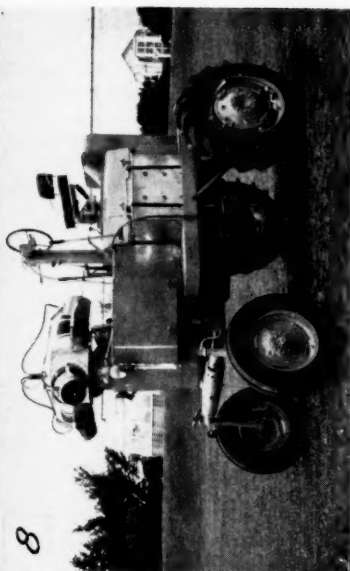


FIG. 8. Cornell experimental concentrate row-crop machine with rotating outlets, 5 to 20 gallons per acre.



FIG. 9. Low gallonage-low pressure sprayer, 20 to 50 gallons per acre.

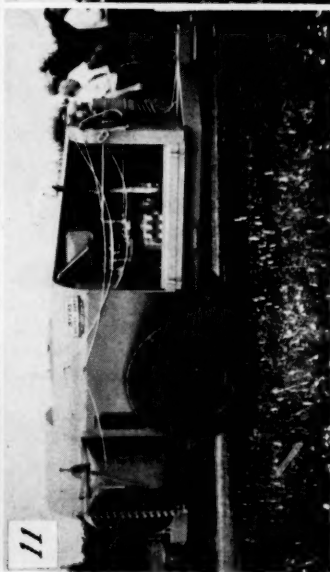


FIG. 11. Myers Field Crop Concentrate sprayer with oval air outlet, 50 to 100 gallons per acre.

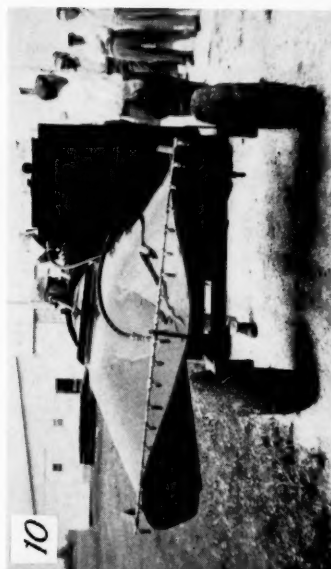


FIG. 10. U. S. Department of Agriculture, Toledo Laboratory experimental row-crop machine with unique air outlets.

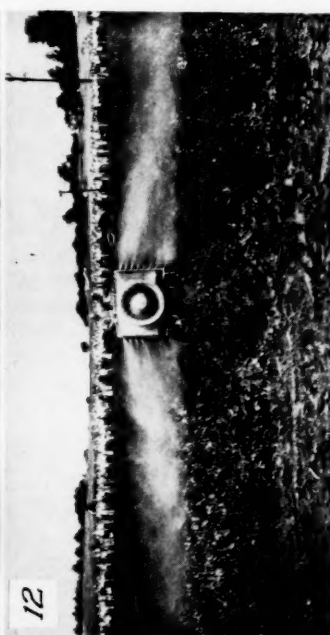


FIG. 12. Bean Speedaire semiconcentrate row-crop machine, 50 to 100 gallons per acre.

The wide use of concentrate and semiconcentrate equipment for pest control cannot be attributed alone to the mechanical developments. The discovery of new and more effective insecticides and fungicides have contributed considerably to this advancement. In most commercial operations the growers are obtaining somewhat poorer coverage than they did with dilute sprayers. The secret of success seems to lie in the speed of application, thus a more timely treatment, and in the efficiency of the toxicant which does not seem to require the high deposit and the evenness of distribution of the earlier chemicals.

The literature indicates clearly that in some respects air blast spraying has now reached the same point that high pressure spraying had in 1940. That is, we cannot go on solving the problems by building larger machines with more air blast. Progress lies in the direction of more efficient application of the power we are now using through a better understanding of the factors involved in getting the toxicant from the tank to the plants.

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ECOLOGY OF FOREST INSECTS¹

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In this review of forest insect ecology, recent literature between 1952 and the first part of 1955 is covered. 1952 was selected as the starting date because prior to that year three publications cover the subject reasonably well. These are *Insect Enemies of Eastern Forests* [Craighead (1)], *Insect Enemies of Western Forests* [Keen (2)], and *Principles of Forest Entomology* [Graham (3)]. Only a few especially significant publications prior to 1952 will be mentioned.

A very complete review of Canadian forest insect work between 1948 and 1953 was presented before the Sixth Commonwealth Entomological Conference in London, July, 1954 [Prebble (4)]. This report includes a bibliography of 151 titles, some of which are available only as unpublished reports.

Because the field of forest entomology must in its very nature rest upon an ecological foundation it is difficult to establish a boundary line separating the strictly ecological aspects of the subject from those parts which have relatively little ecological significance. For purposes of this review the following general rules have been followed. An attempt has been made to cover significant contributions that deal directly with the interactions of insects within a population; with their associates, both plant and animal; and with their habitat. Publications concerned with insecticides and chemical control have been excluded except in instances where ecological implications are brought out. Likewise articles have been omitted that deal exclusively with taxonomy, species lists, and with life history studies unrelated to environmental factors.

Even after these exclusions there remain numerous, worthwhile publications that have been omitted because of space restrictions. The selection has been made with hesitancy and fear that personal interest might weigh too strongly in the selection of those publications which are particularly pertinent. Length of an article does not necessarily indicate its significance, and some of those considered important are very brief. Preference has been given to publications containing a comprehensive list of references.

The results of a considerable amount of important research in forest insect ecology from Forest Insect Laboratories of the U. S. Department of Agriculture cannot be included because they are disseminated for administrative use and not for publication. This work has been concerned with a variety of forest insect pests, dendroctonus beetles, the spruce budworm, the larch sawfly, and numerous others.

¹ The survey of the literature pertaining to this review was completed in May, 1955.

INDUSTRIAL FOREST ENTOMOLOGY

During the past few years large timberland operators have come to realize the importance of protecting their lands against insects and disease, and a few of them now employ full time forest entomologists. Interestingly enough, the desirability of applying ecological principles to the control of forest pests by silvicultural or utilization practices occupies a prominent place in the thinking and recommendations of these industrial forest entomologists as illustrated by a report by Argetsinger, Flink & Prielipp (5). Although this publication contains little original material, it is an important indicator of a recent trend in the application of ecology to forest entomological problems.

OUTBREAKS AND THEIR CAUSES

The history of past outbreaks of forest insects and the events leading to the development of outbreaks have always stimulated interest. Written history since the settlement of North America has given the impression that outbreaks of the larch sawfly, spruce budworm, and other pests arose simultaneously over vast areas. Such a pattern does not harmonize with that which is known about more recent cases. Populations usually have increased in especially favorable centers, and during a pre-outbreak period of several years, have spread out. Ultimately all favorable types in a locality became involved. Establishing the pattern that was followed by unrecorded or inaccurately recorded forest insect outbreaks through measurement of relative width of annual rings has received considerable attention since Craighead, Swaine and Bailey many years ago demonstrated the ring pattern characteristics of spruce budworm injury. Such a study reported by Blais (6) traces the development and decline of an outbreak of the spruce budworm, *Choristoneura fumiferana* (Clem.), that occurred in the latter half of the nineteenth century in Ontario, based on the ring pattern of surviving white spruce *Picea glauca* (Moench) Voss. Supporting evidence is provided by the age class distribution of balsam fir, *Abies balsamea* (L.) Mills, currently growing in the locality. Their age corresponds approximately with the time of the pre-historic outbreak that destroyed most of the mature balsam fir then standing.

Similarly, in another locality Turner (7) presents evidence from annual rings of a still earlier outbreak in Ontario. Especially interesting is the observation that recent outbreaks studied intensively have followed much the same pattern of origin and spread as some previous outbreaks [Blais (6)].

Influence of climate and weather.—A number of very important publications have appeared in recent years concerning the influence of climate and weather upon forest insect populations. Outstanding among these are certain papers by Wellington (8, 9, 10). Continuing his earlier work on the same subject, which describes the meteorological principles concerned and applies these principles to some insects, i.e., the spruce budworm, *Choristoneura fumiferana* (Clem.) and the forest tent caterpillar, *Malacosoma disstria* Hbn., he demonstrates that a definite pattern of atmospheric circulation is likely to precede outbreaks of these insects. A strong northerly atmospheric com-

ponent characterizes the years preceding outbreaks of the spruce budworm, and a strong southerly influence the years preceding forest tent caterpillar outbreaks. In short, dry summers are conducive to the former, and moist summers the latter.

The influence of radiation upon the temperature of insectan habitats is discussed in considerable detail by Wellington (8), presenting a mass of original data and summarizing literature on the subject of temperature effects upon microhabitats.

Henson & Shepherd (11) point out that the temperature within a leaf is by no means identical with that of the surrounding atmosphere. Henson, Stark & Wellington (12) show that as a result of vertical distribution of temperatures prevailing during cold winter weather, lowest temperatures are most likely to occur either on valley floors or at the top of slopes, depending on type of atmospheric circulation prevailing at any given time. Therefore, mortality of the lodgepole needle miner, *Recurvaria milleri* Busck, resulting from low temperature is most likely to occur at these high and very low zones. The reservoir for reinfestation then would most likely be on the middle slopes.

Hawbold (13) in discussing climate and the birch dieback concludes that shift in climatic conditions combined with other ecological factors have been primarily concerned in the dieback.

Variations in weather conditions from season to season frequently result in peculiar seasonal population patterns. For example, Rose & Blais (14) report such an unusual condition. In 1952 instead of a single period of emergence from hibernation unseasonably warm weather followed by a more normal cool period caused a double emergence of overwintering spruce budworm larvae, a part emerging early and the remainder following the cool period. This resulted later in the season in considerably more than usual difference in stage of development between the older and younger larvae comprising the population. In the same paper he presents data showing the influence of temperature upon emergence over a six-year period.

Mortality of overwintering insects as a result of extremely low winter temperature has been observed in the case of the lodgepole needle miner (15) and the Engelmann spruce beetle, *Dendroctonus engelmanni* Hopk. Terrell (16) reports that the parasite and predator complex associated with the Engelmann spruce beetle, although injured by cold weather, suffered relatively less than did the bark beetles. Thus, the influence of these beneficial insects may be indirectly amplified by the effects of low winter temperature. Barter (17) reports a widespread reduction in 1948 of beech scale, *Cryptococcus fagi* (Baer), in New Brunswick as a result of severe cold weather. Other similar references are scattered through current literature. The interrelation between temperature effects and the effects of predators and parasites emphasizes the importance of adopting a comprehensive ecological approach to forest insect problems.

Following his 1951 report on the responses of three forest defoliators to

polarized light, Wellington (18) reports further observations on the light responses of three closely related tent caterpillars (19). This work emphasizes the fact that the reaction to light may be reversed from positive to negative or vice versa under conditions of high temperature, that photo reactions are sometimes complicated by changing response between instars, and that some species make use of natural polarization patterns when travelling over a surface.

Reversal of their photoresponses by the forest tent caterpillar larvae when exposed to high temperature directs this tentless caterpillar to the shade, whereas the tent-building caterpillars that usually respond negatively and therefore gather in their tents during the day, react positively, when heated, and leave the tent. Such responses have, of course, a high survival value. Other recent publications that contribute materially to an understanding of forest insect reactions to light, heat, and humidity appear in the bibliography (20, 21).

In his comparative study of the ecology of three species of sawflies, belonging to the genus *Diprion* or *Neodiprion*, in Belgium, Elens (22) reports that in general the species of this genus exhibit a higher resistance to desiccation than other genera partly because of the type of cocoon construction. Mortality rate of the eonymhs exhibited an inverse trend with the relative humidity. The incubation period required to hatch the eggs of these sawflies showed that *Neodiprion* (*Diprion*) *sertifer* (Geoffr.), the species with the most northerly distribution, required the shortest time for incubation. This Elens interprets as indicating a special adaptation to a northern climate.

The influence of low temperature on the possible northern distribution of certain forest insects, notably the gypsy moth, *Porthetria dispar* (L.), and the European pine shoot moth, *Rhyacionia baoliana* (Schiff.), has long been recognized. Confidence in the validity of previous conclusions that the former will be limited in its northward distribution by minimum winter temperature of -25°F . and the latter -18°F . is shaken by two reports. The pine shoot moth has extended its range in Michigan beyond the temperature limits previously established (23), and a Russian worker, Kozhanchikov (24), reports that gypsy moth eggs when protected from autumn rains are able to withstand temperature ranging between -31° and 49°F . with not more than 5 and 10 per cent mortality.

Weather, of course, influences water levels which in turn affect swamp inhabiting species, especially the larch sawfly, *Pristiphora erichsonii* (Hartig). Lejeune, Fell & Burbridge (25) conclude that flooding of swamps, especially when it occurs in the late summer, will destroy the submerged larch sawflies within cocoons. In the spring the insects are more resistant to submergence except after transformation to the pupal stage.

Weather conditions may affect a population adversely when the normal rate of development is retarded by cool weather. Tinker (26) reports such an instance when during one year, as a result of cool weather, the second

generation of the box elder bug was still incomplete at the end of the season. This operated as a natural control agency. In comparing recorded populations of this insect with weather conditions, he found that high populations were correlated with relatively high seasonal summation of temperature above 20°C.

Intraspecific population influences.—Competition for food or space has frequently been referred to as the cause responsible for the collapse of forest insect outbreaks. Also movements within a locality from centers of high density to less densely populated spots, or local mortality resulting from overpopulation may change the population distribution pattern within a community. Robert (27) reports such a situation with a *Melolontha melolontha* L. infestation, local spots overpopulated one season were occupied by only a sparse population the next.

Franz (28), on the basis of published European records, concludes that outbreaks of forest insects may be brought to an end as a result of inbreeding in dense populations. He postulates that even without the influence of starvation, natural enemies, or unfavorable weather, a dense population will degenerate and ultimately collapse. According to his hypothesis, each individual possesses in its genetic constitution recessive characters which have low survival value. In a dense population the manifestation of these undesirable qualities will increase with each generation as a result of greater opportunity for inbreeding. Ultimately almost all the offspring of a generation may exhibit one or more of these previously hidden qualities. Because they are unadapted to survive, the majority of the population will succumb and the outbreak will be brought to an end.

ECOLOGICAL ASPECTS OF FOREST INSECT SURVEYS

Since 1947 there has been an increasing interest in annual inventory of forest pests and their damage. This interest is evident not only in the public agencies which have long conducted such work, but also among timberland owners and operators. As a result, much effort has been devoted to the development and evaluation of new techniques for measuring insect populations either directly or in terms of their injurious effects. An important objective of these annual inventories is, of course, to anticipate outbreaks that may require the application of control measures; however, the annual census of various insects is also an essential part of many research programs.

In a discussion of surveys in general, Orr (29) divides them into classes according to the purpose for which each is intended: detection, reconnaissance, appraisal, area hazard, and damage surveys. Each of these survey types requires the measurement of populations in varying degree of detail, and all carry ecological implications. Space will not permit complete discussion of publications concerned with forest insect surveys, but even without such discussion, a list of subjects covered will give some idea of the scope of the work. Sequential sampling (30, 31), population sampling and analysis

for various species such as lodgepole needle miner (32), poplar foliage insects (33), larvae of the winter moth (34) are a few samples of the kind of quantitative work involved in annual surveys.

Questions pertaining to the reliability of sampling procedures invariably arise when any measurements of forest insect populations are made. Often, especially when the populations are at low density the problem of obtaining adequate sampling is especially difficult. Schwenke (35) points out the importance of stratifying samples by forest type, site and age class of the trees, each stratified group to be handled separately.

Roegner-Aust (36) comments that predictions of nun moth outbreaks based upon pupal counts alone are not reliable because of the variability in larval mortality in the succeeding generation. He suggests that by rearing larvae from field-collected eggs under ideal conditions in the laboratory before the normal time of hatching, that an advance estimate of the mortality to be expected from disease and constitutional weakness can be made. It seems likely that similar procedures might be applied to other similar problems.

Prediction of injury before it becomes conspicuous is important if direct control measures are to be planned in advance, as they should be. In this connection Benjamin (37) reports the use of tree form to determine when inconspicuous injury reaches a critical stage. The form is expressed as a decimal ratio of lateral-terminal growth of the trees. Marked deviations from the normal ratio occur on red pine trees injured by the Saratoga spittle bug, *Aphrophora saratogensis* (Fitch), prior to the appearance of "flagging," which is ordinarily the first gross sign of injury that is easily observed.

The use of radioactive isotopes has received considerable attention and, although still in the experimental stages, promises to be useful in studying the local movements of individual insects [Sullivan (38); Fang (39)].

REGULATION OF POPULATIONS BY SILVICULTURE

There is a distinct increase of interest in developing and applying silvicultural practices designed to reduce the amount of land occupied by hazardous forest types. Turner (7) points out the possibilities and limitations of this approach as applied to the spruce budworm, *Choristoneura fumiferana* (Clem.). Although in certain respects his observations are in conflict with earlier observations, nevertheless he urges application of preventative silvicultural practices in localities where accessibility permits.

The application of silvicultural practices requires constant attention to economics, as pointed out by Morris (40). The construction and maintenance of a road system in order to bring about the necessary degree of accessibility and the management costs involved will have to be counterbalanced by benefits from reduced average insect losses. "... the effectiveness of control by silviculture and management will not be fully known until one or more future outbreaks have been experienced." Nevertheless, there

seems to be a rather general feeling among foresters and many forest entomologists that silvicultural practices afford one promising approach to the control of many forest insects. These viewpoints and specific practices for control are summarized by Prebble (41) and other Canadian forest entomologists for a number of important forest insects and disease conditions, including birch dieback; spruce budworm, *Choristoneura fumiferana* (Clem.); larch sawfly, *Pristiphora erichsonii* (Htg.); hemlock looper, *Lambdina fiscellaria lugubrosa* (Hulst.); and the mountain pine beetle, *Dendroctonus monticolae* Hopk. The necessity of harmonizing direct methods of forest insect control by chemicals with the preventative biological and silvicultural methods are emphasized by Balch (42).

The influence of stand composition is brought out by Turner (7) and Kinghorn (43). Generally diversification either by species or by age class results in a reduction in the amount of insect injury.

The use of fire as a silvicultural tool has value in the regulation of both injurious insects and disease-causing organisms. Ground fires that destroy forest litter can control some vectors of virus diseases (44), and barkbeetles that hibernate in the litter. Lekander (45) reports 90 per cent control of *Ips typographus* in Sweden by spring burning prior to beetle emergence from hibernation. Similar control might be expected with some other species of *Ips* such as *I. perturbatus* and *I. pini*. Baldwin (46) suggests practices designed to prevent barkbeetle attack, and the use of benzene hexachloride as a silvicultural tool. By spraying undamaged trees surrounding those already attacked their infestation may be prevented and at the same time emerging beetles that alight on the sprayed surfaces may be killed.

ELEVATION OF BIOLOGICAL FORMS TO SPECIFIC RANK

A number of forest insects have long been separated biologically into two forms, but taxonomically have been considered identical. In contrast some appear to be identical biologically and morphologically but are designated as separate species chiefly upon the basis of geographic distribution. This has caused considerable confusion.

In recent years, cytological and genetic studies have provided fundamental information which has resulted in separating several biological forms taxonomically. For example, the form of *Agrilus* feeding on the trunk and large branches of aspen, formerly considered to be a form of *Agrilus anxius*, Gory, has been described by Freeman (49) as *Agrilus liragus*. It was first established as a separate species on the basis of cytological studies (47, 48); morphological distinctions were later observed.

Similarly, the two forms of the spruce budworm, *Choristoneura fumiferana* (Clem.), long considered to be a single species, are now recognized as taxonomically distinct (49, 50, 51). The pine form is known as *Choristoneura pinus* Free. Presumably these closely related species have developed one from the other as a result of biological and temporal isolation (52, 53).

Further studies of this sort may not only demonstrate distinctions between species, but may, on the other hand, show closer relationships than currently recognized, or actual synonymy between species now thought to be distinct.

INSECTS IN TRANSMISSION OF TREE DISEASES

The part played by insects in the transmission from tree to tree of fungi, bacteria, viruses, and other causes of diseased conditions has received attention from forest pathologists and entomologists whenever an important outbreak of tree disease has occurred. Currently special attention has been devoted to possible vectors of oak wilt.

Insects associated with the mycelial mat of the fungus formed beneath the bark, nitidulid beetles particularly, and which later are attracted to, or make, wounds suitable for infection are thought by Craighead (54) to be the chief, although not the only, agency responsible for transmitting the disease from tree to tree. He feels that the vectors must be very inefficient carriers of the inoculum or the disease would spread far more rapidly than it does. Griswold (55) reports transmission of oak wilt by the pomace fly.

Hypoxylon canker, one of the most serious fungal parasites of aspen, is frequently associated with insect injury (56). In this association the insects probably do not often themselves transmit directly the disease-causing organism, but merely create favorable infection courts.

The transmission of the Dutch elm disease by the elm bark beetles continues to receive attention although no noteworthy publications have appeared since 1952 except notes concerning the spread of the introduced vector mentioned in the next section (59). Phloem necrosis of elm is apparently transmitted only by a single species of leafhopper, *Scaphoideus luteolus* Van D. (70).

SPREAD OF FOREST INSECTS

The rate at which insects are capable of moving from centers of infestation or extending their range provides excellent material for discussion and argument. However, specific information concerning these matters is usually quite inadequate, based on reports of discoveries in new localities. Three important forest pests have recently been discovered beyond their former known ranges. Odell (57) reports on an outbreak of the gypsy moth in Michigan and describes an apparently successful effort to eradicate this dangerous pest. Benjamin & Underwood (58) discovered Swain jack pine sawfly, *Neodiprion swainei* Midd, in Sauk County, Wisconsin; and Beckwith (59) publishes the first official record of the small European elm barkbeetle, *Scolytus multistriatus* Marsham, in Wisconsin.

The movement of insects from centers of heavy infestation commonly involves spread by normal flight. Occasionally mass flights are observed. Henson (60) briefly summarizes conclusions based on 19 observed flights of the spruce budworm recorded at the Forest Insect Laboratory at Sault Ste. Marie, Ontario. He finds that some characteristics of their flights are quite

constant. (a) "They always take place in the evening or early night." (b) "The farther the area of deposition from infestation, the later at night the flight is observed." (c) Passage of a "cold front" followed the deposition of the moths. (d) If rain preceded the deposition it was brief. He concludes that when these so-called mass flights occur the insects, when in flight locally, are carried high in the air by the updraft of an approaching storm and deposited at a considerable distance, battered but still active.

The methods by which scale insects spread from tree to tree has been studied by Rabkin & Lejeune (61). By placing tanglefoot traps at varying distances and in different positions in reference to trees heavily infested by *Toumeyella numismaticum* (Pettit and McDaniel) they demonstrated that the scales in the crawler stage were carried from place to place by wind. They consider that transportation by insects, birds, or mammals, although possible, is relatively unimportant.

REARING INSECTS

Before thorough studies can be made of the reactions of an insect to its environment it is obviously desirable that ways of rearing it under controlled conditions be developed. The problem of providing suitable food is often difficult, especially for insects that require foliage or growing tips in a specific stage of development. Also the creation of a favorable habitat, and the problems involved in directing larvae to their food, call for ingenuity. An excellent illustration of how a good technique has been developed for rearing the spruce budworm, an insect that has been especially difficult to handle under laboratory conditions, is the work of Stehr (62).

By freezing expanding tips of balsam fir they can be kept until needed, thus providing food at any season, and by taking advantage of the insects' reaction to physical conditions their movements may be directed within the small dishes used for rearing. Freezing of fresh food for use at seasons when it is not naturally available obviates to some extent the necessity of developing synthetic rearing media that have hitherto received considerable attention (63).

RESULTS OF FOREST INSECT DAMAGE

The injury caused to trees by the attack of forest insects may be expressed in loss of increment, degradation of the wood, or in the deterioration of killed standing timber. The ecological coactions that are involved may include only those between the insect and the tree, or they may also include complex series of reactions between many kinds of organisms that live in the dead and dying trees. These coactions are usually considered from the economic rather than the ecological viewpoint, but nevertheless they have ecological implications.

No attempt has ever been made to study the entire complex of insects and other arthropods, fungi, bacteria, and other microorganisms that inhabit dying and dead trees. however, individual groups have been studied.

For example, Belyea (64, 65) reports upon the insects associated with the death of balsam fir, *Abies balsamea* (L.), following defoliation by the spruce budworm, *Choristoneura fumiferana* (Clem.). In these articles the author indicates the part played by each species in the series of events leading to the death of the trees. Similar work has been conducted in the northeast and is mentioned anonymously in a report of the Northeast Forest Experiment Station (66). This latter work is concerned chiefly with the rate of deterioration after death of balsam fir and two species of spruce.

In the Northwest deterioration rates of beetle-killed Douglas fir have been observed by Wright & Wright (67). The authors found a close association between the sap rots and the beetle tunnels. They believe that the insects were responsible for introducing the decay-causing organisms into the trees. Deterioration of western hemlock following defoliation is attributable in large part to *Fomes pinicola* (Sw.) according to Engelhardt (68).

CONTROL OF FOREST INSECTS

The ultimate objective of the forest entomologist is to bring about the regulation of insect populations so that their numbers will be kept below the threshold of economic injury. This may be accomplished by a variety of means, both direct and indirect. In the following pages will be reviewed some of the more significant contributions that are pointed directly or indirectly toward the regulation of populations. The over-all problem of protecting forests against insects and fungi is well presented by Prebble & Bier (69). The principles and problems discussed, although directed specifically toward Canadian problems, are generally applicable and illustrate well the many angles from which forest insect control may be approached.

Ecological effects of insecticides.—The ecological effects of insecticides have provided material for much discussion and some arguments. References to the increase in populations of aphids, scale insects, and mites following the application of DDT are numerous but for the most part repeat that which has been said before. An especially interesting situation has developed on the University of Illinois campus as reported by English & Decker (70). Spraying with DDT to control the leaf hopper, *Scaphoideus luteolus* Van D., vector of phloem necrosis, and later to control the European elm bark beetle, *Scolytus multistriatus* Marsham, was followed by a marked increase of scale insects. This situation has necessitated the addition of a scalecide to the spraying treatment. English & Tinker (71) also refer to the increase of aphid and mite populations following the spraying of elms with DDT.

Some efforts to avoid the deleterious effects of insecticidal applications while at the same time benefiting by their use was discussed editorially by Michelbacher (72) who advises the use of lowest practicable dosage and careful timing of treatments in order to minimize undesirable effects.

Some workers are looking toward the use of systemic insecticides to avoid undesirable effects and increase specificity of the treatments. In dis-

cussing internal medication of plants for controlling insects, Anderson (73) points to the effectiveness against some sucking insects of quassia extract and parathion, applied to the soil, taken up by the roots, and translocated to above-ground parts of potted plants. Although much current literature has reported experiments with systemics on field crops, little has appeared in print on how these materials may be used on trees. A fertile field has been opened that promises high return for effort expended.

The importance of timing the application of insecticides is mentioned in literature over and over, and it may be assumed that timing must be based upon a knowledge of insect habits and responses to environmental conditions. That such knowledge is often inadequate is brought out by Cannola *et al.* (74) who reports success in controlling the European pine shoot moth during one season and no control the following year using the same material and dosage. The failure was presumably attributable to poor timing of the latter application, but why it was poor was not explained.

It seems apparent that many workers now recognize the importance of applying insecticides so as to cause a minimum of disturbance to the biotic complex. Therefore we may expect some important contributions during the next few years on this subject.

Parasites and predators.—Publications since 1952 which discuss insect parasites and predators of forest insects are so numerous that space limitation precludes the possibility of mentioning all of them in this review. Therefore, an attempt has been made to select a few representative papers that emphasize the interrelations between parasites or predators and their forest insect hosts or prey.

Dowden (75), speaking before the Ninth International Congress of Entomologists, presented his ideas of the place of natural control in forest practice as compared with other control procedures. He emphasized the desirability of introducing parasites into areas where they do not occur with the objective of improving the effectiveness of the parasite complex.

In discussing the numerical relations that commonly exist between forest pests and their natural parasites, Thalenhorst (76) points out that parasites and predators often fail to increase at a rate corresponding with that of their hosts in times of outbreak. This situation he, in part, explains by the fact that host and parasite may be effectively separated as a result of their differential response to environmental factors. Some of these factors tend to bring insects together, localizing their distribution, whereas others tend to disseminate the insects widely. Obviously if the host population is spreading while the parasite is prevented from so doing, the two may in part become separated, thus reducing the potentialities of control by the parasite. The host-parasite relation is a complex and dynamic phenomenon involving both time and space.

Sellers (77) expresses agreement with the conclusions of Clausen relative to the time factor in biological control. His long experience substantiates the claim that if a parasite introduced into a new environment is to be fully

effective it will manifest its ability, near the point of liberation, within three years after introduction. The influence of less-effective parasites depends chiefly upon the number of consecutive years that the host continues to be numerous. To the reviewer such generalizations, although often correct, seem dangerous because of the many variables involved.

One illustration of how a parasite, *Mesoleius tenthredinis* Morl., which soon after its introduction became effective against its host, the larch sawfly, *Pristiphora erichsonii* (Htg.), and later became of little consequence, is reported by Muldrew (78) and discussed by Lejeune & Hildahl (79). The sawfly population today exhibits a high degree of resistance to the parasite. Presumably such resistance was not exhibited immediately following its introduction when it was reported to be an effective control agency. Resistance in this instance is accomplished through the encapsulation of the parasite eggs in the body of the host.

Reeks (80) in discussing the establishment of a number of introduced parasites of the European spruce sawfly points out that *Exenterus clavipennis* (Thompson) increased rapidly at points of liberation. He suggests that its over-all effectiveness at the release point may have been reduced by its dispersal into adjacent areas which occurred at the rate of at least eight miles per year.

The ability to seek out and parasitize its host when a low population of the host prevails is, of course, one of the desirable qualities for a parasite to possess. Also the ability to disperse rapidly from points where it has become established is to be desired. Both of these qualities are exemplified to a high degree by an introduced parasite of the European larch casebearer, *Coleophora laricella* (Hubn.). This small parasitic wasp, *Agathis pumilus* (Ratz.), was released in 1937 and became established at two points near Ann Arbor, Michigan. No other releases were made in the Lake States. By 1951 it had spread across the Straits of Mackinac to Ste. Ignace, 300 miles north, and to the west around the southern end of Lake Michigan for about the same distance, into southern Wisconsin [Webb (81, 82)]. Near Ann Arbor 60 per cent of the overwintering casebearer larvae were parasitized in spite of very low host density.

An interesting competitive coaction between *Agathis* and another introduced parasite of the casebearer, *Chorysocharis laricinella* (Ratz.), was earlier reported [Graham (83)]. The latter is not present in southern Michigan but is established in the upper peninsula of Michigan (81). By preventing pupation of the casebearer larvae it has parasitized, *Agathis* causes host larvae to be available for the spring generation of *Chorysocharis*. The result is that this three-generation species is an effective limiting influence only when aided by *Agathis*. The *Agathis* population is, of course, correspondingly reduced, since within the body of the host larvae it cannot compete with *Chorysocharis*.

The distribution of parasites in strata within a tree crown is brought out in an article by Jaynes (84). Parasitism of the spruce budworm by *Apanteles*

and *Glypta* is greater relatively in the upper than the lower parts of the crown. Samples taken from mid-crown approach the average for a tree as a whole. Such facts are often overlooked in sampling insect populations and thus avoidable errors are introduced.

The assessment of mortality caused by parasites presents such difficulties that results of population measurements are usually expressed in terms of per cent of mortality occurring in a given stage. Morris & Miller (85) point out the danger of misinterpretation inherent in this practice. When comparison from year to year is desired, the significance of the percentages is affected by differences between years in other mortality factors operating on earlier stages of the host. They suggest the development of life tables that will express all mortality factors in common terms, giving illustrations of such tables. Miller (86) compares techniques for determination of "apparent parasitism" and points out that some of the difficulties encountered in calculating the effect of parasitism during the larval stage can be eliminated by estimating host mortality as the product of apparent parasitism and host density at the time when the parasite starts to kill the host. This involves the determination of apparent parasitism . . . , a knowledge of parasite development in relation to host development, and the population density of the host larvae at certain critical periods

The role played by predatory species in limiting the increase of forest insects is probably far more important in the ecology of forest insects than the number of publications on this subject would seem to indicate. The difficulties inherent in quantifying predatory influences account in part for this neglect. This same thought is expressed by Morris (87) in the concluding sentence of an article concerned with the population of small forest mammals. He says, "It is concluded that much work of a fundamental nature remains to be done before practical population techniques will be available to the ecologist." Nevertheless, he was able to draw valuable general conclusions concerning the numbers of small mammals in the areas studied.

Buckner (88) in discussing the influence of small mammals upon the larch sawfly, *Pristiphora erichsonii* (Htg.), states that the greatest amount of predation by small mammals occurs in September when the mammalian populations are at a peak.

Thompson (89), in reporting on the Bermuda juniper scale brings out that, contrary to usual opinion, certain coccinellids are selective in their food preferences and do not feed habitually upon all insects with which they are associated.

Predatory activities by birds has seldom if ever been accurately evaluated. Clearly, however, the population limits set by the territorial requirements of most perching birds during the breeding season restricts their potentiality for controlling outbreaks of forest insects. Cheshire (90) records little increase in the over-all bird population following a greatly increased population of spruce budworm. However, eaters of larvae did increase while other birds decreased. No change was measurable in the territorial require-

ments of the two warblers that became especially abundant, i.e., the Bay breasted warbler and the Tennessee warbler. Dowden *et al.* (91) attempted to measure the effect of birds by comparing the budworm population on plots from which birds were removed by shooting, with a plot on which they were undisturbed. The greater number of larvae on the former as compared with the latter indicated that birds were exercising an appreciable effect. One conclusion reached is that birds should be of greater value in light than in heavy infestations.

Virus-caused diseases of insects.—The use of viruses for the control of forest insects promises soon to pass beyond the experimental stage. Dowden (92), Benjamin *et al.* (93), and Bird (122) report on the successful use of a virus to control the European pine sawfly, *Neodiprion sertifer* (Goeff.), and Bird (94) and Bird & Whalen (95) describe in detail the effects of the virus upon the sawfly larvae. Other articles on viruses of specific forest insects have been published recently by Graham (96), Bird & Whalen (97), and Bird (98). One article by Bergold (99) deserves special mention. In it he reviews the literature on insect viruses, including viruses affecting forest insects.

Influence of physiological condition of the attacked tree.—The comment is frequently heard that trees in good health are less subject to insect attack and injury than are those in poor physiological condition. It is less common to find references to the specific physiological conditions responsible for the differences. Conditions that encourage attack by barkbeetles are perhaps the exception. Kraemer (100) has related osmotic pressure of the phloem fluids to susceptibility of trees to four European barkbeetles. *Dendroctonus mecans* Kreg under experimental conditions was able to attack healthy trees with osmotic pressure 11 to 12 atmospheres in the upper parts and 10 in the trunk, but preferred 5.0 to 8.5 atmospheres in nature. The other beetles tested showed preference for osmotic pressure at a level indicating them to be secondary species, although he points out that, in times of drought, pressure may be so reduced as to permit them to attack the trees.

Thalenhorst (101), in his studies of a pine sawfly in Europe, *Gilpina frutetorum* F., a species which lays eggs singly, found that many eggs failed to hatch as a result of the flow of resin from the oviposition wounds in the needles. He concludes that this response of the healthy trees limits the increase of this and other sawflies with similar oviposition habits. Since drought has a tendency to reduce the resin flow, it would naturally follow that outbreaks of *G. frutetorum* would be associated with drought. Examination of weather and outbreak records discloses that this is true.

Inouye (102) has related moisture content of the outer two centimeters of the sapwood to the suitability of trees and logs for barkbeetles. He found a direct relationship to exist between the ability of certain barkbeetles to attack healthy trees and their response, determined experimentally, to percentage of moisture in the sapwood.

In an attempt to determine the reasons that certain trees are selected for attack by *Dendroctonus ponderosae* Hopk., while others are not, Mogren (103) compared paired trees, one attacked and the other not attacked, grow-

ing together under similar conditions. He found that trees of higher vigor, classified according to Keen's ponderosa pine classification, were significantly less likely to be attacked than those in lower vigor classes. Also, trees in higher vigor classes were significantly more likely to throw off an attack than those in the lower vigor classes.

That balsam fir, *Abies balsamea* (L.) Mill., bearing large numbers of staminate flowers support greater numbers of the spruce budworm, *Choristoneura fumiferana* (Clem.), than trees with few flowers, has been clearly demonstrated by Blais (104) who has made a detailed study of this matter. The physiological conditions characteristic of old age result in the production of an abundance of staminate flowers and are responsible, therefore, for producing conditions conducive to outbreaks of this insect.

MISCELLANEOUS FOREST INSECTS

Numerous publications dealing in whole or part with the ecology of forest insects have appeared since 1952. However, space does not permit a critical review of all of them. Therefore, only brief comments will be made concerning some that seem particularly significant.

McGugan (105) in a comprehensive study of the needle mining habits of the spruce budworm, reports that this habit is, contrary to earlier ideas, typical of the species. He also presents conclusive evidence that the budworm passes through six larval instars, not seven as some suspected. Detailed studies of scale insects are not too numerous and therefore the excellent study by Cumming (106) of the pine leaf scale, *Phenacaspis pinifoliae* (Fitch), is a welcome contribution. Also, the life history study of the spruce gall midge [Smith (107)], *Phytophaga piceae* Felt, provides valuable information concerning a little-known group of insects.

The pitch nodule maker (pitch blister moth), *Petrova albacapitana* (Busck), has become increasingly abundant in jack pine plantations in the Lake States and eastern Canada. Formerly considered to be of little significance economically, it is today responsible for excessive damage locally. Therefore, Turnock's study (108) of this insect is of special interest.

A brief note by Underwood (109) points out that there is a biennial cycle of injury in the case of pine leaf chermes, *Pineus pinifoliae* (Fitch), in New Brunswick. This is apparently attributable to the alternation of hosts which characterizes the life cycle of the species.

The periodical cicada, *Magicicada septendecim* (L.), outbreaks always attract attention. Graham & Cochran (110) and Asquith (111) report on the emergence of this species in Maryland and Pennsylvania respectively.

An injurious wood insect, the old house borer, has been causing increasing damage in the eastern United States, where it is locally injurious. Becker (112) comments on this insect in Massachusetts where it is common over the eastern half of the State. A distribution map of the species may be found in the May, 1955 number of the *Cooperative Economic Insect Report* (113).

It is an interesting and encouraging fact that the population of the

gypsy moth has been so reduced by a combination of natural control factors and spraying operations, that it has been necessary to go to Europe for a supply of female moths needed for production of the sex-attractant bait. Holbrook (114) describes the European collection program. It need scarcely be mentioned that the attractant bait is used to apprehend the presence of the insect in localities where its population is too low for detection by other means.

Less attention than their importance justifies has usually been directed toward the study of insects that injure tree seeds, but several reports concerning these insects have recently appeared. Radcliffe (115) presents an appraisal of the damage caused by the Douglas fir cone moth, *Barbara colfaxiana* (Kearf.), pointing out that the yield may be reduced by more than 38 per cent. Tripp (116, 117) gives a detailed account of the life cycle of a spruce cone inhabiting maggot and the spruce seedworm, *Lespeyresia youngana* (Kft.), an insect which in some seasons may virtually destroy the seed crop.

The Engelmann spruce beetle, *Dendroctonus engelmanni* Hopk., between 1939 and 1951 destroyed approximately 4.3 billion board-feet of timber in Colorado and has prompted intensive study of the ecology of the beetle. Massey & Wygant (118) summarize the results of these studies. Unlike most species of *Dendroctonus*, the Engelmann spruce beetle requires two years to complete development, and the adults, after leaving their pupal cell, seek hibernation quarters under the bark near the base of large trees.

Woodpeckers were especially active in feeding upon the beetles, in some places reducing the beetle population by 98 per cent. Other causes of beetle mortality were parasites, insect predators, and low winter temperature. The outbreak of the Engelmann spruce beetle developed in a windfall, as did most other known outbreaks of this insect. Connola *et al.* (119) present an unusual analysis of the insect populations in windthrown trees in the Adirondacks but fail to bring out clearly the ecological implications.

A series of outbreaks of the Douglas fir beetle, *Dendroctonus pseudotsugae* Hopk., had their origin in windthrown trees in western Oregon and Washington. An excellent report on this insect and its associates has been prepared by McCowan & Rudinsky (120), forest entomologists employed by the Weyerhaeuser Timber Company. In this report the life history and natural control factors are emphasized. Among other things, the deleterious effects of associated fungi upon the developing brood of the beetles is brought out. When the growth of fungi is able to advance faster than the larvae, the brood dies.

The galls produced by *Adelges abietis* Linn. on Norway spruce, *Picea excelsa* (Linn.), have long attracted attention and prompted much speculation as to the character of the stimulus producing them. Plumb (121) has thoroughly reviewed the literature on gall formation in general and has studied specifically the morphology and development of the spruce gall. He was unable to isolate or otherwise identify the substance responsible for stimulating gall development, but was able in a single case to bring about

complete gall development by combining feeding by the gallicolae with injections of salivary secretions of the overwintering fundatrix.

In concluding this review of literature on the ecology of forest insects, one is impressed with the broad scope of the subject, the many angles from which it is being attacked, and the real progress that is being made. The risk that some important contribution has been overlooked is directly proportional to the breadth of the subject, but it is hoped that any omissions attributable to oversight will not be too serious. The selection of articles for comment has, of necessity, been arbitrary and not always easy, but has been guided by the rules set forth in the first part of this chapter. It is hoped that the personal interests of the author are not too apparent.

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SOME RECENT ADVANCES IN APICULTURAL RESEARCH¹

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In view of the limitations that inevitably have to be placed on the amount of space in this volume that can be allotted to a review of recent work on apiculture, I have considered it to be more satisfactory to attempt to deal fairly fully with a few selected topics rather than to try to cover a wider field even more briefly. It is for this reason that no mention has been made of the very considerable advances that have recently taken place in our knowledge of the aetiology and treatment of several of the more important diseases of bees, or of much new work on foraging behaviour and pollination, or, indeed, about many other topics. I hope, however, that sufficient has been said on the few topics which have been chosen to indicate some of the main trends of thought and research today.

EFFECTS OF ANAESTHETICS ON ADULT BEES

Carbon dioxide has for years been used as a cheap and efficient anaesthetic for adult bees. However, in 1950 Ribbands (1) showed that one of the results of a few minutes anaesthesia with carbon dioxide is to cause bees that have hitherto been foraging for pollen to stop collecting this kind of food and to gather nectar instead, sometimes even from the same flowers from which they have previously collected pollen. Nitrogen has a similar effect to that of carbon dioxide, but after chloroform anaesthesia, no alterations in behaviour have been observed. Ribbands, therefore, concluded that it is probable that these changes in behaviour are the results of a short period of acute oxygen lack, and Simpson (2) has shown that this may be produced either by a temporary deficiency of oxygen in the atmosphere or by interference with the processes involved in oxygen uptake.

Although carbon dioxide must, therefore, be discarded as an anaesthetic in most behaviour studies it has been found to be most useful when inseminating queens instrumentally (3) as its use leads to early oviposition. It has also been used by Mackensen (3) to cause virgin queens to lay unfertilised drone-producing eggs at an early age, and the semen of the resulting drones has even been used to inseminate their mothers, thus obtaining rapid inbreeding (4).

These effects of carbon dioxide anaesthesia, together with Ribbands' (1) observation that it will cause young bees to forsake household duties and

¹ The survey of the literature pertaining to this review was completed in March, 1955.

start foraging at an earlier age than similar control bees, suggest that it may cause physiological ageing of adult queens and workers. However, Ribbands (1) has further shown that the life expectation of bees so treated is not significantly different from that of similar untreated individuals.

Whereas carbon dioxide anaesthesia leads to early oviposition in the case of queen honey bees, Fyg (5) has shown that it actively inhibits ovary development in workers under conditions in which such development would otherwise occur. Furthermore, he has found that it also tends to inhibit such activities as comb building and food storage.

Important claims have recently been made by Gontarski (6) for the practical merits of certain fumes, produced by dropping ammonium nitrate crystals on to the glowing embers of fuel in a beekeeper's smoker, as an anaesthetic for worker honey bees. Gontarski believed that the active principle was nitrous oxide and claimed that anaesthesia with it causes worker bees to lose their memory for a hive site, thus enabling a beekeeper to move a colony even a short distance in an apiary without any tendency for the bees to desert the new site for the old one. He further claimed that such anaesthesia did not alter the comb building, brood tending, or foraging behaviour of the bees concerned.

A number of other workers, including Brunskill & Rankin (7), have supported some of these claims but no satisfactory experimental data appeared until 1954 when Ribbands (8) published the results of experiments which clearly showed that neither anaesthesia with pure nitrous oxide nor that with the fumes produced by the use of ammonium nitrate in the manner described by Gontarski (6), causes bees to lose their memories for hive sites. Furthermore, both Ribbands (8) and Simpson (2) found that Gontarski's (6) claim that there is no danger of giving bees an overdose of these fumes is incorrect, and that unless care is taken an overdose can easily be given. Simpson (2) further showed that the efficacy as an anaesthetic of the fumes produced by adding ammonium nitrate to the fuel in a beekeeper's smoker is certainly not attributable to nitrous oxide but possibly to hydrogen cyanide (or cyanogen) or, more probably, to some other unidentified component.

Contrary to the statements of Gontarski (6), anaesthesia with "ammonium nitrate smoker fumes" as with carbon dioxide or nitrogen (1), causes foragers to stop collecting pollen and accelerates retrogression of the pharyngeal of young bees (2). It might, therefore, be considered probable that a single dose applied to a whole colony would upset the division of labour amongst its workers, but no such detrimental effect has been observed (2).

Despite the fact that "ammonium nitrate smoker fumes" do not have the effects originally claimed for them there is no doubt that when carefully used they can serve as a useful anaesthetic for whole colonies. Anaesthesia thus induced can be employed for such purposes as the volumetric determination of the number of adult bees present, provided that some alteration in behaviour can be tolerated. If no alteration in behaviour can be allowed then chloroform is still probably the most useful anaesthetic known.

COLONY ODOUR

In 1919 von Frisch (9), experimenting with a number of boxes, found that food-seeking worker honey bees tended to be attracted to those which had very recently been visited by other honey bees. Thus it appeared probable that the latter had left a perceptible sign, probably a scent, behind them. Later, in 1923, von Frisch reported that honey bees sometimes expose their Nassanoff glands when flying around, or feeding at, dishes containing abundant, concentrated, sugar syrup and that the scent thus disseminated serves to attract other bees to the sources of food (10). In 1926 von Frisch & Rösch (11) reported seeing flower-visiting honey bees exposing their scent producing glands in this way. Nobody else appears to have reported seeing foragers of *Apis mellifera* Linnaeus exposing their scent glands when visiting flowers, but Butler (12) frequently saw workers of both *Apis indica* Fabricius and *Apis florea* Fabricius doing so in Ceylon.

Von Frisch & Rösch (11) were also the first to publish satisfactory experimental evidence indicating that every colony of honey bees possesses an odour which differs from that of other colonies, and which is distinguishable from other colony odours by the bees of the colony to which the scent-producing bees belong. These results have been thoroughly confirmed and extended by Kalmus & Ribbands (13) who have produced evidence that the food shared by the members of a colony, which may be expected to differ slightly in the proportions of its floral constituents from that of other colonies (14), plays a major part in determining colony odour. This does not, however, eliminate the possibility that certain other factors, such as absorption of scent (15) from flowers and from the hive atmosphere (16), play some part, if only minor ones, in determining the body odours of individual bees and hence of the colonies to which they belong. The fact that a strange bee can within two hours apparently acquire sufficient of the odour of a colony into whose hive she has strayed to cease to be molested by the guard bees and, it would appear, to become accepted as a member of the colony whose hive she has entered (17), tends to lend support to the view that absorption of scent plays a part in determining colony and individual bee odour.

The production of a common odour shared by all the members of a colony is likely to depend upon extensive food sharing (13) amongst the adult members of the colony. Whilst food sharing has long been known to occur, the extent and speed with which very small quantities of food, collected by very few bees, are shared has only recently become apparent thanks to the work of Nixon & Ribbands who used a radioactive tracer technique (18). Other workers, including Alpatov & Saf'yanova (19) and Istomina-Tzvetkova (20), have also made valuable observations on this extremely important aspect of bee behaviour, which, as several authors have recently pointed out (18, 21 to 24), plays a most important role in colony organisation and integration.

CLUSTERING

Although stray bees are particularly strongly attracted towards any

cluster of workers in which a queen of any kind is present (23), the presence of a queen is not essential to cluster formation (25). Indeed, in 1950, Lecomte (26, 27) showed that when approximately 100 or more worker bees are scattered at random in a darkened box they will, within about three hours, form a single, compact, queenless cluster. If, however, less than about 50 bees are placed in the box at room temperature they will remain scattered or only form small, temporary, groups each composed of not more than three or four individuals. It appears, therefore, that a certain minimum number of bees must be present before the stimuli which result in cluster formation become effective. Free & Butler (24) subsequently obtained data which support this conclusion.

Lecomte (26) concluded that one of the stimuli which attracts stray individuals towards a cluster is olfactory and originates in a bee's abdomen, and that stray bees are also attracted by the vibrations produced by a cluster. He did not, however, suggest that the Nassanoff gland, whose scent is very attractive to other worker bees (10 to 13, 28), might be the source of this olfactory stimulus, nor was he able to demonstrate that either an olfactory stimulus alone, or a vibratory stimulus alone, was sufficient to cause stray bees to join a cluster. Therefore Lecomte (26) concluded that both these stimuli must be in operation simultaneously, each reinforcing the effect of the other, if scattered bees are to be caused to cluster together.

This problem has recently been further studied by Free & Butler (24) who have shown that each of the following stimuli, all of which are produced by clustering bees, are individually capable of attracting stray bees and causing them to cluster near the source of the stimulus: (a) scent from the Nassanoff glands, (b) vibration, and (c) warmth.

The vibratory stimulus, which in some experiments (24) was produced by bees who were shut up inside a tin and in others by a simple mechanical vibrator within a tin, is probably perceived by means of sensillae in a bee's legs (29) via the object on which the individual is standing rather than by air-borne vibrations (30). The sensillae responsible for perception of the heat stimulus have been shown by Heran (31) to be mainly, but not exclusively, concentrated in the last five segments of the antennae.

Free & Butler (24) further demonstrated in the laboratory that bees are attracted towards other actively moving bees by sight alone, and Kalmus (32) has shown the importance of this visual stimulus in attracting bees to sources of food in the field. He also found, as the work of Hansson (30) had suggested, that bees do not appear to respond in any way to air-borne vibrations produced by other bees.

Hungry bees have been shown to prefer to join clusters of bees from whom they can obtain food (24). Indeed a group of well-fed bees appears to be more attractive to stray individuals than does a similar group of hungry bees. As this occurs even under conditions when the stray bees are unable to have any physical contacts with the well-fed bees, it seems probable that well-fed bees are more active and produce more powerful scent and vibratory stimuli than hungry bees (24).

The extensive food sharing which continually takes place amongst the adult members of every honey bee community (18) and the worker bee's desire for queen substance (23), together with a worker bee's need for a resting temperature which is usually higher than that of the external environment and is found in a cluster, are all factors which are important in the maintenance of a cluster once it has formed (12, 24).

LAYING WORKERS

It has been known for a long time that if the queen is removed from a colony of honey bees and the bees subsequently fail to rear a new queen to replace her, the ovaries of some of the workers develop from their normal rudimentary condition and unfertilised eggs are often laid. In general it seems to be true to say that no queen of any kind must be present if the ovaries of workers are to develop quickly and to any considerable extent (33 to 36). However, it has been found that at certain times, such as during the week or two preceeding the issue of a swarm (37) or in a colony in mid-winter (38), the ovaries of a proportion of the workers sometimes develop to a small extent even though a queen is present.

Although reports do exist of worker bees mating with drones (39, 40), it is most improbable that insemination ever occurs as no spermatheca is present in the worker (41, 42). In consequence, any eggs laid by worker bees are unfertilised and normally give rise only to drones in accordance with the well-established theory of Dzierzon (43). Occasionally, however, colonies which have been queenless for long periods of time and contain functional laying workers, but have had no fertilised eggs or young worker larvae from which to rear replacement (emergency) queens, somehow acquire queens (12, 41, 44). It has been suggested that the workers of such queenless colonies have reared their new queens from larvae from fertilised eggs stolen from other, queenright, colonies (45), but it seems much more probable that they have in fact been reared from female larvae produced from unfertilised eggs laid by workers of the queenless colonies themselves. It has been shown that such unfertilised, normally male-producing, eggs do exceptionally give rise to female larvae (46) from which queens can be reared.

Several writers have attempted to explain the development of the ovaries of some of the workers in colonies which have lost their queens by supposing that, in the absence of an adequate number of larvae to feed, the nurse bees possess a surplus of brood food (pharyngeal gland secretion) which somehow results in ovary development (47, 48). However, as Ribbands (22) has pointed out, the work of Hess (34) indicates that such a theory cannot fully explain the observed facts. From the data obtained subsequently by Müssbichler (49) and other workers (38, 50) it has become clear that if the ovaries of a group of young worker bees are to develop it is essential for protein to be available to the bees concerned either in their current diet or as reserves within their fat-bodies, and, further, that no queen of any kind shall be present (33 to 36).

It appears probable, therefore, as suggested by Butler (23), that the

worker honey bees of a colony normally obtain a substance from the body surface of their queen which inhibits development of their ovaries. This hypothesis is, of course, somewhat similar to that of Hess (34) who suggested that an ovary inhibiting substance might be collected from the excrement of the queen. Pain (51), on the other hand, after reviewing the data of Müssbichler (49) reached the conclusion that worker honey bees produce a "fertility substance" which they normally feed to their queens, and which accounts for the great fertility of the latter. She supposed that in the absence of a queen the workers are compelled to retain their "fertility substance" with the result that their ovaries develop and many of them become functional laying workers. Later, however, Pain (35) discarded this view in favour of the hypothesis suggested by Butler (23) namely that worker honey bees obtain from their queens a "queen substance" which is an ovary-inhibiting factor. Recently, de Groot & Voogd (36) have succeeded in obtaining effective extracts of this "queen substance" both in ethyl alcohol and in acetone.

Butler (23) came to the conclusion that queen substance is somewhat fugitive and probably forms a part of the waxy covering of a queen's body. He further suggested that, although his data demonstrated that it is present on all parts of a queen's body, it is particularly abundant on her abdomen. Pain (35) and de Groot & Voogd (36) have, however, clearly shown that it is particularly concentrated, not on a queen's abdomen, but rather on her head, and, furthermore, that it is much more stable than Butler (52) had supposed. As Butler (23) has pointed out, it seems possible that similar ovary-inhibiting substances exist in ants and termites. Indeed Bier (53) has recently demonstrated their existence in several species of ants.

CONTROL OF QUEEN PRODUCTION

If a colony of honey bees loses its queen, the worker bees often appear to have become aware of the fact within an hour or less, and signs of restlessness and colony disorganisation become apparent but quickly disappear if the queen is returned or a suitable new queen is given to the bees. If, however, the colony is left without a queen for a few hours, the exact length of time depending on the strain of bee and possibly some other factors (23), the workers modify one or more worker brood cells containing young female larvae to form emergency queen cells and thus take the steps that are necessary to rear a new queen for themselves.

The problem of how the bees realise that their queen is no longer with them and begin to take steps to replace her has attracted considerable attention. It seems impossible to believe that every worker bee in a colony can make direct contact with her queen often enough (i.e., at least once every hour or so in many small colonies) to remain aware of her continued presence in the hive. Various other explanations have, therefore, been suggested.

The hypothesis that a special queenly odour exists which permeates the hive atmosphere and thus keeps all the bees aware of the presence of their queen is an explanation that has, and probably still is, widely held amongst

beekeepers. However, as long ago as 1814 Huber (54) showed that this could not be the correct explanation. He found that if the queen of a colony, together with some of her workers, was separated from the other members of her colony by means of a perforated partition through which scent, but not bees, could readily pass, and which prevented the bees who were separated from their queen from making physical contact with her, emergency queen cells were soon built by the latter, but not, of course, by the bees actually with the queen. In 1952 Müssbichler (49) described similar experiments in which she obtained similar results. She also found, however, that even if the bees that were separated from a queen by means of a wire-gauze partition could make physical contact with the bees who were with the queen on the other side, queen cells were started by the queenless group of bees. This result does not at first sight appear to agree with those obtained in similar experiments by Butler (23), who found that queen cells were not as a rule started if the workers of the two groups could make contact with one another through the screen. Further experiment has shown, however, that if two groups of bees that are separated from one another by a simple perforated screen are both well supplied with food they will not make much contact with one another through the screen (24). If, on the other hand, the bees of one or another group are hungry then they will solicit food from the members of the other group and make physical contact with them (23, 34). The fact that the bees on both sides of the perforated screen in Müssbichler's (49) experiments were well fed, whereas the bees in some of Butler's (23) experiments were not, explains the different results obtained.

[The importance of using wire-gauze of large mesh, through which bees can readily make contact with one another, when making cages for introducing new queens to colonies has recently been re-emphasized (55); and the possibility of encouraging the bees of the recipient colony and the queen to make contact with each other through the wire-gauze by refraining from providing the queen with food in the cage is well known (56, 57)].

If one discards such hypotheses as those which suggest that the members of a colony are kept aware of the presence of their queen because her scent permeates the hive atmosphere, or that the bees may assume that their queen is still with them unless they are informed that she is not by means of special sounds produced by certain members of her colony who happen to be the first to become aware of her absence (48), one must suppose that the workers of a colony are normally kept aware of the continued presence of their queen indirectly, by those bees who have recently been in contact with her somehow informing other members of their colony of this fact (23). Müssbichler (49) amongst others has adopted this latter view, basing her opinion on the results of some experiments in which she found that when a queen was caged amongst a group of her own workers these bees did not exhibit any signs of queenlessness provided that they could actually make contact with their queen.

Butler (23) carried out experiments in which queens were caged in various

ways so as to vary the extent to which their workers could gain access to them. The data thus obtained show that unless the bees had fairly ready access to their queens they built emergency queen cells, and the conclusion was reached from these and other results that the members of a honey bee colony normally obtain some substance from their queen which inhibits them from attempting to rear further queens, provided that a sufficient quantity of the substance is available. In further experiments, in which only restricted areas of queens' bodies were exposed to their bees, it was shown that the bees could obtain this "queen substance" from all parts of the body surface of a queen. Furthermore, both virgin and mated queens produce it, though the readiness with which worker bees distinguish between these two types of queen is probably dependent upon differences in the qualities of the queen substances produced by them.

Evidence was also brought forward (23) which suggests that a small proportion of the members of a colony obtain queen substance directly from their queen by licking her body and subsequently share it widely with other members of their colony in regurgitated food. The work of Park (58) and of Nixon & Ribbands (18) has shown how rapidly and extensively even small quantities of food are shared amongst the members of a colony, and this food sharing habit appears to be sufficient to ensure quick and adequate distribution of this substance.

The bees who obtain queen substance directly from the queen are some of those who turn to face her, and form a circle around her, as she moves over the combs. The bees who form this "court" are young ones (59) who merely happen to be near the queen when she stops walking about (60) and, besides licking her, they feed her and frequently examine her body very thoroughly with their antennae.

It seems highly probable that the queen substance which inhibits ovary development in worker honey bees (35, 36), effective extracts of which have been obtained in several organic solvents (35, 36), is also the substance which controls queen production (23, 35). If this is the case one might expect that some development of the ovaries of workers would occur when the amount of queen substance available to the members of a colony falls below a certain threshold and queen cells are built. Perepelova (37) has, indeed, shown that this does occur.

Butler (23) concluded that anything which interrupts, or seriously reduces the availability of queen substance amongst the workers of a colony results in removal or reduction of the inhibitory effect, and either the construction of emergency queen cells or toleration of the presence of eggs or larvae in queen cell cups. Thus he was able to demonstrate that when a queen begins to fail and her workers commence to make preparations to rear a new queen to supersede her, her production of this substance has become reduced below the threshold necessary to inhibit this action on the part of her workers. The data of Nolan (61) appear to show a correlation between a reduction in the rate of egg laying of a queen and her supersedure. J. Simpson

(personal communication) has suggested that it is possible that the reduction in queen substance output, which has been shown to occur in the case of a queen who is being superseded (23), leads to a reduction in the amount of brood food (pharyngeal gland secretion) which her workers give her, i.e., the bees become less able to appreciate that she is a queen (at any rate with regard to this particular attribute) and so give her less brood food, with the result that she can only produce a small number of eggs.

Furthermore, it seems probable that either a deficiency in the amount of queen substance available, or, perhaps, a breakdown in its collection and subsequent distribution [possibly because the queens are at this time being pushed about a great deal by the workers (62)] plays an important part in the phenomenon of swarming.

It is not yet clear, as Wigglesworth (63) mentions, whether queen substance itself acts directly upon the metabolism of worker bees or whether it merely serves as a trigger mechanism which results in the release within the body of a recipient bee of a hormone which is the actual inhibitor. If queen substance is indeed only a trigger mechanism it seems quite probable that other releasing mechanisms, possibly psychological ones, may exist.

CASTE DETERMINATION

In 1845 Dzierzon (43) published his famous theory that, after she has mated, a queen honey bee is able to lay two kinds of eggs, unfertilised ones which give rise to drones only and fertilised ones which give rise to female bees only, i.e., workers and queens. This explanation of the origin of male and female honey bees has been widely accepted for many years now. Recently, however, Mackensen (46) has shown that a very small number of females are sometimes produced parthenogenetically in some races of *Apis mellifera*.

The ability of worker honey bees to rear both queens and workers from fertilised eggs has long formed the basis of practical queen rearing by beekeepers, and there is no possible reason for supposing that differentiation of the queen and worker castes in honey bees is determined genetically in the same sort of way as it is in bees of the genus *Melipona* (64). It follows, therefore, that caste differentiation in the honey bee is almost certainly determined by the feeding of the female larvae, although there is just a possibility that the different planes in which the larvae lie in queen and worker cells respectively may have some influence on the result (12).

In 1888 and 1889 von Planta (65, 66) published the results of his investigations on the chemical composition of the food of queen, worker, and drone honey bee larvae and reached the conclusion that whereas for the first three days of larval life they are all three fed on a similar kind of food [shown by strong circumstantial evidence and some serological work (67) to be produced in the pharyngeal glands of adult worker bees], on or about the fourth day of larval life the food of workers and drone larvae becomes diluted by the addition of honey. However, von Planta (66) also stated that the queen larvae are given much more food than worker larvae. Köhler (68) also

decided that pollen and honey is added to the larval food of workers and drones about the fourth day of larval life, but Nelson & Sturtevant (69) observed the presence of pollen grains in the food surrounding worker larvae that were only three days old and, therefore, concluded that the change in the diet of larvae destined to become workers occurs earlier than the fourth day of larval life. The analyses of the food of worker and queen larvae carried out by Melampy, Willis & McGregor (70) shows that differences in composition are apparent as early as the second or third day of larval life. The results of earlier investigations of larval respiration by Melampy & Willis (71) also indicate that by the time a larva which is destined to become a queen is between two and three days old her metabolic rate is considerably higher than that of a worker larva of the same age. It can reasonably be concluded, therefore, that worker and queen larvae begin to be fed differently by the time they are two days old, a conclusion which supports the opinions of Zander (72), Snelgrove (73), and others that worker larvae that are to be transferred into queen cell cups, by beekeepers who are rearing queens, should be as young as practicable.

Von Planta (65, 66), Nelson & Sturtevant (69), von Rhein (74), and other observers have noted that the food upon which older worker larvae are fed usually contains numerous pollen grains, the contents of which the larvae are undoubtedly capable of digesting (75), whereas few, if any, pollen grains are found in the food of queen larvae of any age. Simpson (75) has recently considered the significance of the presence of pollen in the food of worker larvae and has shown that they receive a very variable quantity of pollen in their food in summer, and sometimes none at all in winter. He has also shown that a growing worker larva probably receives less than one-tenth of her nitrogen requirements from the pollen contained in her food, and he concluded that pollen is not an essential constituent of the food of worker larvae and that the scarcity of pollen usually observed in the food of queen larvae [Haydak (76) reports the occasional presence of traces of pollen in their food] cannot account for their differentiation from worker larvae. Indeed, Haydak (76) has even gone so far as to suggest that the pollen grains that are found in the food of worker larvae have probably only got there accidentally from the mouthparts of nurse bees who have recently been feeding on pollen. As Simpson (75) points out, however, since the carbohydrate content of the food of worker larvae of different ages varies (65, 66, 76), it is probable that the sugary material which the nurse bees regurgitate from their honeystomachs, to add to the larval food, contains variable amounts of pollen which has not yet been transferred into their ventriculi by means of the mechanism described by Bailey (77).

In another hypothesis, which has been put forward in an attempt to explain caste differentiation, it has been supposed that slight differences occur in the quality of the brood food (pharyngeal gland secretion) fed to the larvae of queens and workers (78), but the actual results of the analyses that have been carried out by various workers (76, 79, 80) do not agree with one an-

other. This has led Haydak (76), who, like Ribbands (22), has reviewed the whole subject of caste determination in the honey bee very fully, to conclude that brood food varies in composition quite fortuitously and that such variations as occur cannot be responsible for caste determination. Furthermore, Kuwabara (81) has found that worker bees of the same age feed both queen and worker larvae, and Lindauer (82) has recorded that a given nurse bee will feed a series of larvae of very different ages one after the other. Unless, therefore, nurse bees are able at will to vary the quality of the brood food which they give to different larvae this hypothesis appears to be untenable.

Having decided that the quality of the food was unlikely to be the responsible factor in caste determination, Haydak (76) considered that it might be attributable to differences in the quantities of the essential nutrients supplied to and consumed by worker and queen larvae respectively. He pointed out that until about the third day of life all larval honey bees in prosperous colonies receive large amounts of food, but that, subsequently, whilst the queen larvae in their large cells continue to be supplied with more food than they can eat, the worker larvae in much smaller cells only receive relatively small quantities of food from time to time. Probably as a result of these feeding regimens the queen and worker larvae grow at approximately the same rate for the first day or two of larval life, but, subsequently, whereas the queen larva, surrounded by an abundance of food, continues to grow very rapidly, the much more frugally fed worker larva grows much more slowly. Haydak (76) pointed out that even after her cell has been sealed a queen larva continues to feed upon the surplus of brood food in the bottom of her cell, whereas a worker larva, having no such source of food in her cell, cannot feed any further once her cell has been sealed, and actually loses weight.

In order to test this hypothesis Haydak (76) removed the larvae from queen cells that were either just about to be sealed or had just been sealed, so that they could not eat any more food. Most of the larvae which he treated in this way died in the pupal stage, but he reported that seven of these pupae possessed worker rather than queen characteristics, whilst several others appeared to be intermediate between queens and workers. Nine adults were produced, all except one of which were normal queens, the exceptional individual being intermediate between a queen and a worker. Haydak reported that the average initial weight of those of the larvae which developed into queens was 14 per cent greater than that of those which developed into individuals with worker or intermediate characteristics.

From these results it certainly appears likely that continuous liberal feeding of those larvae who are destined to become queens plays an important role in their differentiation. However, von Rhein (74), who fed female larvae in the laboratory very liberally with brood food taken from queen cells, failed to obtain the queens one would have expected if Haydak's (76) hypothesis is correct. Indeed his failure to produce queens in this way led von Rhein (74) to suppose that some unstable, hormone-like, differentiating

substance is fed by workers to those larvae which are destined to become queens, and that this fugitive substance had been destroyed, or lost during storage, in the brood food with which he fed his larvae.

Very recently Weaver (83) has reported the results of some experiments in which he fed young larvae, taken from worker cells, on an abundance of freshly collected brood food taken from queen cells containing larvae of approximately the same ages. Under these circumstances queens were produced. On the other hand when similar larvae were fed on abundant food which had been collected from queen cells and stored for some time only large worker bees were produced. These results clearly do not support the conclusion reached by Haydak (76), as the experimental larvae were continuously supplied with a superfluity of food so that quantitative starvation could not have been the determining mechanism. On the other hand they strongly support the conclusion of von Rhein (74) that some substance, or substances, contained in the food fed to queen larvae controls their differentiation into queens, and that at least some essential part of this substance is either highly labile or else is no longer available to larvae after the food has been either exposed to air, or stored in a refrigerator, for some time.

It seems probable that in order that a given female larva may give rise to a queen she must continuously receive liberal supplies of fresh brood food, in which the rather labile differentiating substance is present. This conclusion suggests that the practice of priming queen cell cups, into which worker larvae are to be transferred in order to induce bees to rear them as queens, with brood food which has been kept in store is, from the point of view of queen differentiation, more likely to be harmful than helpful.

DIVISION OF LABOUR

For many hundreds of years beekeepers have realised that some sort of division of labour exists amongst the worker members of every colony of honey bees. Indeed the development of such a division of labour became necessary as soon as colonies evolved which consisted of more than a very few individuals. Thus, even amongst the bumble bees, the colonies of many of which only consist of perhaps 30 or 40 adult members, a rudimentary division of labour has been shown to exist (84, 85).

At first it was almost inevitably supposed that a king, and later a queen, bee was responsible for directing the labours of the worker members of the colony and, although the work of Rösch (59, 86, 87), Lindauer (88), and many others has shown that this theory is quite untenable, there are still a few beekeepers who seem to believe in similar theories (89).

The first detailed studies on the nature of the division of labour in honey bee colonies were those of Rösch (59, 86, 87) who, by making use of an observation hive and bees so marked that he could recognise individuals, found that the average expectation of life of a worker bee belonging to a colony which possesses brood is, during the summer months, about 32 days. Another investigator has concluded that the average expectation of a work-

er's life under such conditions is about 21 days and that the maximum is about 45 days (90), and other workers (21, 91) have reported maximum lengths of life of 40 and 51 days. Ribbands (21) records a mean length of life of about 34 days, with a range of between 17 and 40 days. If, however, a colony possesses no brood during the summer months, then the lives of its workers become greatly increased (92); and, during the winter months, worker bees live very much longer than is usual in summer (93 to 96). This increased expectation of life of bees during the winter appears to be correlated with the fact that the fat-bodies of "winter" bees are, at the beginning of the winter, much more highly developed than those of "summer" bees from normal, queen-right, colonies (96, 97). This development of the fat-bodies results from heavy consumption of pollen in autumn (96, 97), when little, if any, protein is required for the elaboration of brood food as breeding is almost, if not quite, in abeyance.

Rösch (59, 86, 87) came to the conclusion that, as earlier investigators had supposed, every worker honey bee carries out a series of different duties in a regular sequence during the course of her life, and that this sequence is approximately as follows: cell cleaning and brood incubating, feeding of the older larvae, feeding the younger larvae, comb building, food ripening and storage as well as other household duties, and, finally, foraging. However, he found that the length of that period of her life which each bee spends performing any particular duty is very variable and, further, that individual bees can undoubtedly adapt their behaviour to meet the day to day requirements of their colonies (87). This adaptability of a worker honey bee has also been well demonstrated by Nelson (98), Ribbands (21), Buchner (99), and other workers.

Other investigators, such as Perepelova (100), Istomina-Tzvetkova (101), and Lindauer (88), have demonstrated how a worker honey bee of a given age will often carry out several of the duties in the sequence mentioned above one after the other in the course of a very few minutes; Sakagami (102) has also published evidence which whilst suggesting that some sequence of duties undoubtedly does occur in the lives of most worker bees, indicates considerable overlapping of several of these duties, so that four main duty periods become apparent.

Although Lindauer (88) watched larvae of different ages being fed on more than a thousand occasions he reports that he never once saw a worker put food directly into the mouth of a larva; instead, the food was placed in a pool around the larva. He also found, as Goetsch (103, 104) had done with ants, that, when capping cells and carrying out other building activities, bees often appear to work against each other rather than to co-operate together. For instance a worker will carefully stick a piece of wax on the lip of a cell, and a few minutes later another bee will as carefully remove it and stick it on again a few millimetres away.

Philipp (105) claimed to have found that worker bees, after cleaning out brood cells from which young bees have just emerged, varnish their interiors

before the queen lays in them again, and Dreher (106) also mentions the use of a varnish for this purpose. However, even though he watched cell-cleaning bees through a binocular microscope, Lindauer (88) was unable to obtain any evidence that such varnishing takes place, and he reached the conclusion that the high polish which is obtained is produced by the bees by laborious work with their mandibles.

From the data he collected from his observations on the behaviour of individual bees Lindauer (88) decided that Rösch's (59, 86, 87) conclusions concerning the apportioning of the different duties are, in general, correct; but, at the same time, he emphasized that it was most striking how, with the exception of the duty of foraging, all the individual bees whose behaviour he observed performed several quite distinct tasks within a short period of time. He further found that a household bee works just as hard at night as she does by day, periods of work alternating with resting periods during which, as he suggests, her pharyngeal glands may be actively replenishing her supply of brood food, or her wax glands may be producing further wax scales.

Gontarski (107) has suggested that any given nurse bee may normally only feed the larvae in a small, circumscribed, portion of the brood area of her colony, but Lindauer (88) could obtain no evidence in favour of this hypothesis and, on the contrary, observed individual bees feeding brood in widely separated parts of the brood nest.

Whereas Rösch (59, 86, 87) assumed that young field bees must themselves have danced before being able to interpret the dances of field bees, Lindauer (88) obtained evidence which strongly suggests that young bees know instinctively both how to interpret the dances of other bees and also how to dance themselves. However, he found that it takes a young bee some time to learn to follow all the gyrations of a dancer accurately enough to interpret her "message." Furthermore, just as Oettingen-Spielberg (108) had found that only a very small proportion of the foragers of a colony go out scouting for new crops, so Lindauer (88) found that very few young bees begin to work in the field before they have learned of the whereabouts of particular crops from the dances of experienced foragers.

Although some beekeepers appear to believe that the division of labour found amongst the foraging bees of a colony is conditioned by their respective ages, Rösch (86) stated that he could find no evidence of the existence of such a mechanism amongst field bees. The extensive data collected by Ribbands (21) and by Lindauer (88) also fail to support this belief. It appears indeed that the type of food bees will collect on their first, as well as on subsequent, foraging expeditions is in some way determined by the current requirements of their colonies. Possibly, as Ribbands (21) has suggested, the extensive food transmission which is continually taking place amongst the members of each honey bee community (18, 58) may somehow lead to individual bees adjusting their activities in accordance with the needs of their community.

Lindauer's (88) observations show that a worker bee spends a great deal of her time patrolling the combs apparently seeking work, and that if she is unable to find work that is suited to her age she endeavours to find something else to do. A bee does not remain idle and should she be unable to find sufficient household duties to do she very soon becomes a field bee.

It seems probable that the apparently complicated and efficient division of labour which is found in every honey bee community is brought about in some such simple manner.

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INSECT TRANSMISSION OF PLANT VIRUSES¹

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Many of our most devastating plant diseases are attributable to viruses transmitted by insects. In the absence of their insect vectors these diseases would be of little importance. Therefore, their control is usually dependent on control of these vectors. But first it is necessary to find out what insects transmit the virus. Of 127 plant viruses listed by Holmes (87) in 1948, the insect vectors were known for 67. Much research has been conducted to determine the relationships between the viruses and their vectors, and some of the landmarks in the progress of these investigations are summarized in this review.

HISTORICAL ASPECTS

Plant virus diseases have been recognized as a distinct class of disorders only since the end of the nineteenth century, when Mayer (122) in 1886, Ivanowski (92) in 1892, and Berjerinck (17) in 1899 demonstrated in mosaic-diseased tobacco an infectious agent, or virus, in the absence of other known pathogens such as bacteria. In 1891, E. F. Smith (149) postulated "some sort of a virus" as the cause of peach yellows, a disease which he could duplicate by grafting but associated with which he could find no trace of bacteria or fungi.

Virus diseases are surely not of recent origin, but before the concept of a virus was developed such diseases were confused with those caused by unfavorable weather or soil conditions or insect attack, or with "degeneration" of plants following long-continued vegetative propagation from divisions, cuttings, bulbs, corms, tubers, buds, scions, or rootstocks. The confusion of virus symptoms with those caused by weather, nutritional imbalance, genetic aberrations, injuries caused by chemicals or insects is still with us, for some such disorders may simulate closely those caused by viruses. The degeneration of vegetatively propagated plants, however, is now generally agreed to result from infection by viruses or by other systemic or recurrent pathogens. One virus disease, tulip breaking, can be traced back to 1576 through published illustrations of the distinctive flower symptoms [McKay & Warner

¹ The survey of the literature pertaining to this review was completed in June, 1955.

(124)]. Others include peach yellows, which was doing damage in peach orchards by 1750, and potato virus diseases, which were serious agricultural problems by 1775 [Bawden (9)].

Research on insects as vectors of a virus causing rice stunt was begun in Japan. In 1895 to 1896 Takata (168) presented evidence that the leafhopper *Deltocephalus* (= *Inazuma*) *dorsalis* Motsch. could transmit the rice stunt virus. Takata's work, published in Japan, remained unknown to the Western World for 40 years. Also in Japan his findings were questioned when *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl. was found to be a more efficient vector, but Fukushi (71) has since confirmed *dorsalis* as a vector. Until Takata's transmission of rice stunt virus by *dorsalis* became known, Shaw's (146) transmission of sugar beet curly-top virus by *Eutettix* (= *Circulifer*) *tenellus* (Baker) was considered to be the first transfer of a plant virus by an insect. Reed's (139) transmission of yellow-fever virus by mosquitoes was the first transfer by an insect of an animal virus.

Research on plant viruses and their vectors progressed slowly for there were only a few workers, and they were pioneers in a new field. Baur (6, 7, 8) demonstrated graft transmission of abutilon mosaic and several other viruses causing variegation, or infectious chlorosis, of woody plants. The mosaic-diseased abutilon, which was prized as an ornamental, seemed dependent on man for survival until Orlando & Silberschmidt (131) in Brazil demonstrated natural transmission of the causal virus by a whitefly, *Bemisia tabaci* (Genn.). During this early period studies on curly top and its insect vector were continued. Storey (161) reported on the maize streak virus transmitted by *Balclutha* (= *Cicadulina*) *mbila* (Naudé), and Kunkel (108) on aster yellows virus transmitted by *Cicadula sexnotata* Fall. (= *Macrosteles fascifrons* Stål.).

However, this period was notable chiefly for pioneer studies on aphid-borne mosaic viruses affecting major crops. Doolittle (55) demonstrated transmission of cucumber mosaic virus by *Aphis gossypii* Glov. and the cucumber beetles *Diabrotica* (= *Acalymma*) *vittata* (F.) and *duodecimpunctata* Oliv. (= *undecimpunctata howardi* Barber). Brandes (30) transmitted sugarcane mosaic virus with *Aphis* (= *Rhopalosiphum*) *maidis* (Fitch). Jagger (93) found lettuce mosaic virus transmissible by *Myzus persicae* Sulz. Doolittle & Jones (56) transmitted pea mosaic virus with *Macrosiphum pisi* Harris. In an intensive study of the virus complex or degeneration diseases in Irish potatoes, in the Netherlands, Ireland, and Maine, the potato leafroll virus and at least two mosaic viruses, each transmitted chiefly by *M. persicae*, and also the spindle-tuber virus, were segregated [Quanjer (136); Murphy (127); Schultz & Folsom (143)]. Johnson (97) in Wisconsin detected potato latent, or X, virus in many American commercial varieties. The first report by Allard (1) of transmission of a plant virus by an aphid has been difficult to evaluate because the identity of his vector, *Macrosiphum tabaci* Pergande, was uncertain and also because aphids have failed to transmit the tobacco mosaic virus from tobacco to tobacco in more recent experiments. Tobacco

mosaic was readily transmitted by aphids from tomato to tomato [Hoggan (85a)]. It is probable that in Allard's experiment the cucumber mosaic virus was transmitted from tobacco to tobacco by *M. solanifolii* (Ashm.) [Walters (177); F. F. Smith (150)].

Although many species of leafhoppers have been established as vectors of plant viruses, aphids continue to outrank all other groups in the number transmitted. Other groups that have been incriminated include thrips, plant bugs, whiteflies, mealybugs, several kinds of chewing insects, and eriophyid mites.

The concept of persistent and nonpersistent modes of plant-virus transmission by vectors, first formulated by Watson & Roberts (184, 185), has proved to be a key contribution, first, as a guide in the technique of handling suspected vectors, second as a means of separating individual viruses from complexes, and finally, to help solve the basic problem of how vectors transmit viruses.

The demonstration that plant viruses are nucleo-proteins, beginning with the studies of Stanley (159) and Bawden & Pirie (12), the recognition of characteristic virus particles under the electron microscope by Kausche, Pfankuch & Ruska (102), and the development of the ultracentrifuge by Svedberg & Pedersen (157) provided techniques and incentives for chemical and physical studies of viruses in the plants and vectors during recent years.

General reference books on viruses and virus diseases include those by K. M. Smith (155), Bawden (9), and Köhler & Klinkowski (106), the last giving references to most of the pertinent literature. Holmes (87) has published a taxonomic list of viruses affecting bacteria, higher plants, and animals. The most detailed account of vectors of plant viruses is that by Heinze (79). Other reviews of insect vectors of plant viruses, or of special phases of this subject, have been presented by Black (24), Christenson & Smith (41), Day & Bennetts (51), Freitag (66), Jensen (95), Leach (110), Maramarosch (119), and Storey (164).

FACTORS IN THE TRANSMISSION OF VIRUSES

Whenever a plant virus becomes of economic importance, three factors are at hand: a reservoir of the virus, a means of transmission, and a susceptible host plant. The susceptible host may generally be taken for granted, for it is lacking only when immune varieties are present or during crop-free periods.

A reservoir of the virus that maintains it over periods of cold or drought is often provided by the crop itself. In perennial crops the virus persists in vegetative propagation from diseased plants. Volunteer plants of the same crop [Evans (59)], other susceptible crops [Bridgmon & Walker (32)], weeds [Severin (144)], or other wild hosts [Posnette (134)] may also serve as reservoirs. Annual plants are sometimes reservoirs for a seed-borne virus. Seeds sometimes serve as the virus source, but seed transmission is uncommon. It is known for only about 5 per cent of the plant viruses and as a

rule occurs in a small percentage of the seeds of a diseased plant [Reddick & Stewart (137); Newhall (128)]. Perhaps 3 per cent of the plant viruses persist in the soil [Allen (2); McKinney (125); Smith (155)]. They form a special group in which vectors, except *Pemphigus lactucae* (Fitch) for lettuce big vein [Thompson, Doolittle & Smith (170)], have no proved role. However, the possibility that insects have a role in the transmission of other soil-borne viruses cannot be excluded.

Some plant viruses do not need a vector to persist and to cause crop injury. The tobacco mosaic virus is readily transmitted during the usual operations of producing a tobacco or a tomato crop [Valleau & Johnson (174)]. The potato latent virus may be transmitted by contact as the plants brush together in the wind [Loughnane & Murphy (112)]. The potato spindle tuber virus is transmitted on the knife in the cutting of seed pieces [Goss (75)]. These three viruses do not require vectors for dissemination, although grasshoppers are capable of transmitting them [Goss (75); Walters (177)]. Stunt virus is transmitted on hands and tools in the production of a chrysanthemum crop; no insect vector is known or needed to account for its behavior [Brierley & Smith (36)]. The tulip-breaking virus, which is typically aphid-borne, may also be transmitted on the knife used for cutting flowers [Van Slogteren & de Bruyn Ouboter (176)]. Cymbidium mosaic and sugarcane ratoon stunting viruses are also transmitted on tools [Jensen & Gold (96); Steindl & Hughes (160)]. However, few of the sap-transmissible viruses are thus transmitted during the normal handling of the crop.

Man himself transmits some viruses through the practice of budding and grafting, methods which differ essentially from those of sap transfer in that union of tissues is involved. The viruses of rose mosaic and rose streak, for which no vector is known, are passed from infected buds to understocks, and from these understocks to healthy buds in the course of propagating roses in the nursery [Brierley & Smith (34)]. Dodders, which are parasitic seed plants, are also capable of transmitting certain viruses [Bennett (14)], but though a useful experimental technique this method is not known to assume importance in nature.

RELATIONSHIP BETWEEN INSECTS AND VIRUSES

There are three phases in the relationship between the insect and the virus in the transmission process: (a) acquiring the virus, (b) carrying it in or on itself, and (c) inoculating it into a healthy plant [Storey (164)].

Acquiring the virus.—The site on which an insect feeds may determine whether it acquires the virus. Some viruses occur mainly in the phloem, others in the mesophyll, and some others are most concentrated in the epidermal cells. Young leaves may carry more virus than older ones [Watson (179)]. Aphids and *Cicadulina mbila* may pick up viruses within a few seconds from the epiderm or mesophyll, whereas longer feeding periods are required to locate and penetrate the phloem tissue [Storey (163)].

Below-ground parts of the plant may be sources of the virus and sub-

terranean insects the vectors. For example, the root aphid, *Pemphigus lactucae*, is a vector of big vein of lettuce [Thompson, Doolittle & Smith (170)], probably because of the unusual dispersal habit of the nymphs [Cutright (46)]. Lily viruses are carried by aphids from bulb scales or sprouts during storage [Smith & Brierley (151)].

Generally both sexes and immature as well as mature insects can acquire and transmit a virus [Bennett (13)]. Notable exceptions are *Thrips tabaci* and *Frankliniella insularis* Franklin [= *schultzei* (Tryb.)], which acquire the spotted wilt virus only while they are nymphs but transmit it as nymphs or adults [Samuel *et al.* (142)]. *Piesma quadrata* Fieb. acquires the virus of beet-leaf crinkle disease as nymphs and adults, but transmits it only as adults [Wille (187)]. The larvae as well as adults of whiteflies can take up the virus of cotton leaf curl, but only the mobile adults transmit it [Kirkpatrick (105)].

Certain leafhoppers can become infective by inheriting the virus itself through the eggs from their maternal parents [Fukushi (70)]. Rice stunt virus has been inherited by *Nephotettix apicalis* for seven generations and clover club leaf virus by *Agalliopsis novella* (Say) for 21 generations without access to diseased plants [Fukushi (72); Black (21)].

Many insects ingest virus while feeding on a diseased plant even though they do not act as vectors. Bennett & Wallace (16) detected virus of curly top in eight nonvector species as long as two to three weeks after feeding.

Storey (162) demonstrated that differences occurred even within a species. He obtained pure races of *Cicadulina mbila* by selective breeding, one of which was able to transmit the virus causing corn streak. The other was unable to infect healthy plants, but if the midgut was punctured with a needle the virus apparently entered the blood stream and could be transmitted. This impermeability of the gut to passage to the virus into the haemocoel was found to be controlled by a single sex-linked Mendelian character. In crossing active and inactive races Storey (163, 164) showed that activity was dominant.

Carrying the virus.—In the transmission of one group of viruses only the stylets are involved; they do not pass through the insect's body. Such viruses may be acquired by a feeding of 10 to 30 sec. and transmitted by a similar feeding period on a healthy plant [Bradley (28); Hoggan (85)].

Maramorosch (119) discussed the transmission of certain other viruses which require many hours, days, or even weeks before the vector becomes infective. While feeding on an infected plant the vector sucks up the virus and ingests it. The virus passes through the gut wall into the blood, from which it can reach the salivary glands and saliva, where it is in a position to be injected back into plants. The virus is retained by the vector for long periods, often for the remainder of its life. This relationship represents a highly specialized adaptation of the virus to the insect; often one or sometimes a few closely related species are found to transmit the virus.

The host preferences of an efficient vector may reduce or prevent the spread of a virus disease. Brierley *et al.* (37) pointed out the danger to tomato

and other crops by infection with tomato aspermy virus, apparently introduced into the United States in chrysanthemum. However, after several years' observations no epidemics have been reported, apparently because the aphids do not feed alternately on chrysanthemum and tomato [Brierley, Smith & Doolittle (37)].

Although spotted wilt virus is generally distributed in dahlia throughout the United States, it apparently does not spread to tomato, lettuce, and other crop plants except in localized areas. Favorable ecological factors are cool temperatures when the concentration of the virus is high, and also the build-up of thrips on dahlias growing in proximity to susceptible crops.

Feeding behavior has been correlated with virus transmission in the xylem-feeding habit of the vectors of Pierce's disease of grape and the phloem-feeding habit of the vectors of potato leaf roll [Huston, Esau & Hewitt (89); Day & Bennetts (51)]. Bawden (9) suggested that irregularity in flow of virus into the saliva might account for the fact that an insect infects only some plants of a series to which it is exposed. Day *et al.* (51a) concluded, however, that the vector may not reach tissue in which the virus can develop every time it feeds.

Regurgitation appears to play a role in the virus transmission of turnip yellow mosaic and squash mosaic [Markham & Smith (121); Freitag (65)].

The ingested virus may come in contact with inhibiting substances in the digestive enzymes. *Macrosiphum solanifolii* is said to be an inefficient vector because it destroys the virus [Watson & Roberts (184)]. Several workers succeeded in infecting leafhoppers by inoculating virus into the haemocoel [Black & Brakke (26); Maramorosch (114, 115)]. Little information is available on the permeability of the midgut to viruses [Waterhouse & Day (178)].

The blood of the vector *Eutettix* (= *Circulifer*) *tenellus* seems to be an important reservoir of the curly-top virus [Bennett & Wallace (16)]. In other vectors the multiplication of virus may very well take place in the blood according to Day & Bennetts (51). They also suggested nonpermeability of salivary glands as a possible barrier to the penetration of a virus introduced into or found in the haemocoel.

Inoculating the plant.—The insect probably introduces the virus into the plant with the saliva, which soon gels and is molded into a sheath. However, Storey (163, 164) concluded that the introduction is discontinuous and independent of the saliva that forms the sheath. On some plants the infection takes place through any part of the plant on which the insect can feed. However, the aleurodid vector *Bemisia* sp. can inoculate cassava mosaic and tobacco leaf curl viruses only into young leaves [Storey & Nichols (165)]. *Cicadulina mbila*, *Circulifer tenellus*, and the sharpshooters transmit corn streak, curly top, and phony peach viruses, respectively, only if they feed in the phloem or xylem [Black (24)].

TYPES OF TRANSMISSION

Storey (164) observed two distinct types of transmission. In one type the

insects fail to infect the first series of exposed plants but in later series begin to infect and may continue throughout their lifetime; in the other type the insects infect the first series of plants to which they are exposed, but few or none in later series. Watson & Roberts (184) proposed the terms "persistent" and "nonpersistent" to designate these two types of transmission.

The persistent virus is characterized by an acquisition feeding period of several hours to several weeks followed by a latent period of similar length before the insect can transmit it. The vector retains the virus for long periods, usually for life. This virus is usually not mechanically transmissible, and there is a high degree of vector specificity. The delay in development of infectivity is characteristic of most leafhopper-borne viruses, all viruses transmitted by thrips and whiteflies, and a few of those transmitted by aphids.

Watson (182) and Kirkpatrick & Ross (103) found great variability among individuals of a vector species and concluded that there was no fixed latent period during which infection could not be obtained provided sufficient trials were made. Bawden's (9) conclusions that a latent period existed regardless of the length of the acquisition-feeding period was explained in part by the disturbance of the vector when transferred manually from one plant to another [Kirkpatrick & Ross (103)]. That the insect can pass the latent period on the source plant during a prolonged acquisition-feeding period was demonstrated by *Capitophorus fragariae* Theob. [= *Pentatrichopus fragaefolii* (Cock.)] with strawberry virus 3 [Prentice & Woolcombe (135)] and also by *Aphis gossypii* with lily rosette [Brierley & Smith (35)].

One virus that seems to be intermediate between the two types is that of swollen shoot of cacao. The mealybug vectors require several hours' feeding to acquire the virus but transmit it in a few minutes' feeding. Further, they lose the virus within an hour if feeding but retain it for 36 hr. if starved [Posnette (134)].

The nonpersistent virus is acquired by the vector during a short acquisition-feeding period and is transmitted immediately during a short infection-feeding period [Watson & Roberts (184)]. The efficiency of transmission is usually increased if the vector is starved prior to the acquisition period. The vector usually retains the virus for only a short time. Most nonpersistent viruses have several vectors, and they are usually mechanically transmitted.

Doolittle & Walker (57) reported that *A. gossypii* could acquire cucumber mosaic virus while feeding for 5 min. on a source plant and transmit it within the next 5 min. while feeding on a healthy plant. Infection rarely occurred in plants to which the aphids were later transferred. However, if the aphids were starved after acquiring the virus, they remained infective for 6 to 8 hr. It was assumed that the virus was carried merely on the mouth parts and was lost with the first feeding [Doolittle & Walker (57); Hoffman (83)]. However, Watson showed, by use of radioactive substance in fluids fed upon by *Myzus persicae*, that the quantity of liquid ingested and the radioactive material injected into plants in subsequent feeding were proportional to the duration of the feeding periods [Hamilton (77)]. She thereby disproved the

idea of transmission by contamination of mouth parts. In further studies with *M. persicae* and *Hyoscyamus* virus 3, Watson (179, 180) showed that the efficiency of aphids as vectors was increased if they were starved for periods up to 1 hr. prior to the acquisition feeding. However, this increase was lost if the subsequent period of feeding on the diseased plant was increased from 2 to 60 min. She also readily obtained infection in the second test plant, but only if the feeding period on the first was of short duration. Aphids that fasted before acquiring the virus retained it on further fasting for several hours, whereas aphids not fasted lost it within 1 hr. The virus was lost by aphids within a shorter time than it was inactivated in expressed juice. On the basis of these studies, Watson (180, 181) suggested that a virus-inactivating substance, possibly a digestive enzyme, is produced in greater quantity while the insect is feeding, but she did not determine how or where the inactivation takes place.

The distinction between persistent and nonpersistent modes of virus transmission [Watson & Roberts (184)] is not as clear-cut as originally assumed. The general concepts are still valid and useful, but a number of intermediate mechanisms are now known with characteristics of persistence and nonpersistence. The concept of a virus inactivator produced at one rate by vectors while feeding but at a lower rate while fasting, although generally accepted [Day & Irzykiewicz (51b)], fails to furnish a satisfactory explanation for vector specificity and for nontransmission of highly infectious viruses. Sylvester (167) reviewed the hypotheses advanced to explain aphid transmission of nonpersistent viruses and offered the following hypothesis: "transmission of nonpersistent viruses by aphids is in essence mechanical and vector efficiency and specificity are due to compatibility factors which are dependent upon specific interactions among the viruses, the saliva of the aphids, and the host plant cells being inoculated." Recent results of other workers agree with this new hypothesis. Bradley (28, 29) found that fasted aphids do not settle down after transfer, but probe the leaf repeatedly and become infective with the nonpersistent virus if they feed for a few seconds only in the epidermal cells, which, according to Bawden *et al.* (11), may contain 16 times as much virus as mesophyll cells. Persistent viruses may be more concentrated in the mesophyll or phloem and require longer acquisition feedings.

MULTIPLICATION OF THE VIRUS IN THE INSECT

Storey (164) presented evidence on the multiplication of the maize streak virus in an insect; when he fed *Cicadulina mbila* on a source plant for only 15. sec., it remained infective for 9 weeks. Kunkel (109) showed that aster yellows virus in *Cicadula sexnotata* (= *Macrostelus fascifrons*) can be destroyed by prolonged exposures to heat, and that if destruction is incomplete during shorter exposures the virus again multiplies when the insect is restored to normal temperatures.

Evidence of multiplication in their insect vectors by passage through

the eggs was obtained for the virus of rice stunt in *Nephotettix apicalis* (Motch.) var. *cinticeps* (Uhl.) for 7 generations [Fukushi (72)], of clover club leaf in *Agalliopsis novella* Say for 21 generations [Black (21)], of wound tumor in *Agallia constricta* Van D. for 7 generations [Black & Brakke (26)], and of corn stunt in *Dalbulus maidis* DeL. & Wol. for 3 generations [Maramorosch (117)]. A fifth virus, rugose leaf curl, in Australia, is transmitted through the eggs of the leafhopper *Nehela torrida* (Evans) [Grylls (76)]. Evidence on the multiplication in *Macrostes fascifrons* Stål of aster yellows virus, which is not passed transovarially, was obtained by serial passage of 10 transfers to adult leafhoppers by mechanical inoculation. Viruses of aster yellows and maize stunt multiply in their respective vectors but not in each other's vectors. [Maramorosch (114, 116)]. They can be transmitted only by the insects in which they are able to multiply [Maramorosch (117, 118, 119)].

In a study of the wound tumor virus, Black (25) found *Agallia constricta* Van D. almost as good a source of the virus as its plant host, sweetclover. The aster yellows virus appeared to be more concentrated in the vector, *Macrostes fascifrons*, than in aster or other host plants.

Although certain viruses multiply equally well and presumably without change in both the insect vector and the plant, Black (23) found that the virus causing wound tumor, maintained in crimson clover for 16 years, could no longer be transmitted by an insect.

Maramorosch (119) postulates that insects constitute a reservoir of certain plant-pathogenic viruses and that plants may often serve as indicator hosts for the viruses harbored by the insects. These viruses were originally insect viruses and through long association between host and virus are completely tolerated by the insect yet cause serious disturbances in the plant. Some could pass indefinitely through the eggs of the vector to the progeny without the necessity of alternate host plants. Others must rely on a life cycle alternating between plants and arthropods. The viruses that multiply in their vectors constitute a link between plant and animal viruses. Their ability to multiply in both plants and animals suggests that they are living organisms.

Although aphids transmit the largest number of plant viruses, multiplication of such persistent viruses as potato leaf roll in *M. persicae* has not been demonstrated. Evidence for multiplication in the vector is also lacking for spotted wilt transmitted by thrips and for cotton leaf curl and other viruses transmitted by *Bemisia tabaci* [Kirkpatrick (105)]. Freitag (63) concluded that curly-top virus does not multiply in the insect vector. He correlated the duration of feeding by *Eutettix* (= *Circulifer*) *tenellus* directly with its ability to transmit the virus. Individual variation in infective ability is attributable to the quantity of virus taken up in feeding and to the age of the insect. In the transmission of curly-top virus and also of tomato big bud virus the latent periods in the respective leafhopper vectors are short, and the ability to transmit extends over a long period [Freitag (63); Hill (82)].

Insects are not harmed by a plant disease virus, and no abnormal change in morphology or physiology of infective individuals has been detected [Black (24)].

TRANSMISSION OF VIRUS COMPLEXES

Three viruses are known that are insect-transmitted only when they occur in plants infected simultaneously with a second virus for which the insect is a vector. Clinch *et al.* (42) discovered that *M. persicae* will transmit potato virus F, the cause of tuber blotch, only if the source plant also contains potato virus A. K. M. Smith (156) found that tobacco rosette has two components, a mottle and a vein-distorting virus. *M. persicae* acquired both components if they were present during the acquisition-feeding period, and it transmitted both viruses after a two-day latent period and retained them for life. If the components were in separate plants, the aphid acquired the vein-distorting virus; it was unable to pick up the mottle virus independently even though it had previously acquired the other component. The relationship between the two components is apparently specific. Smith combined mottle virus with several other aphid-transmitted viruses in suitable host plants, and in no case did the presence of the second virus enable the aphid to pick up the mottle virus.

M. persicae transmitted tobacco ringspot when it occurred in gladiolus simultaneously with bean yellow mosaic [F. F. Smith & Brierley (153)]; the latter, a nonpersistent virus, is readily transmitted by the same aphid. K. M. Smith (156) suggested that the vein-distorting virus may absorb the mottle virus so that both are picked up by the feeding aphid. This would agree with the conditions in the tests by Smith & Brierley (153), where the coiled filiform rods of bean yellow mosaic virus may enmesh the smaller spherical particles of tobacco ringspot virus [Van Koot *et al.* (175)].

TRANSMISSION BY VARIOUS VECTORS

Aphids.—More plant viruses are transmitted by aphids than by any other group of insects [Heinze (79)]. *M. persicae*, one of the most efficient vectors, is reported to transmit more than 50 plant viruses [Freitag (67)]. Most aphid-borne viruses are mosaics. It is estimated that 15 viruses are transmitted by aphids in the persistent manner. These represent a definite minority of aphid-transmitted viruses [Black (24)]. A single aphid is capable of transmitting both persistent and nonpersistent viruses from the same host [Watson (183)].

Leafhoppers.—Leafhoppers are second in importance to aphids in the transmission of plant viruses. Oman (130) listed 69 species of leafhoppers that transmit 26 viruses. Symptoms of viruses transmitted by leafhoppers include yellows, chlorotic streaks, necrosis of the phloem, and tumors. Leafhopper-borne viruses are generally not mechanically transmitted [Black (24)].

Whiteflies.—The whitefly, *Bemisia gossypiperda* Misra and Lamba, trans-

mits cotton leaf curl virus. Both nymphs and adults can acquire the virus, but only the adults transmit it [Kirkpatrick (104)].

Storey & Nichols (165) found that whiteflies could inoculate cassava plants with a mosaic virus only through immature leaves. *Bemisia tabaci* transmits abutilon mosaic virus [Orlando & Silberschmidt (131)].

Mealybugs.—Mealybugs, chiefly *Pseudococcus njalensis* Laing and *Pseudococcus citri* Risso, transmit the virus of cacao swollen shoot [Cotterell (44)]. *P. citri* also transmits tobacco mosaic virus [Newton (129)]. Black (24) suggests that mealybug wilt, long ascribed to a toxin, may be attributable to a virus acquired by mealybugs from resistant pineapples and transmitted to susceptible pineapples.

Thrips.—*Thrips tabaci* Lind., *Frankliniella schultzei* Tryb., and *Frankliniella moultoni* Hood transmit tomato spotted wilt virus [Samuel *et al.* (142); Sakimura (141)]. Pistacia rosette virus is reported transmissible by *Liothrips pistaciae* Kreutz. [Kreutzberg (107)]. The spotted wilt virus is acquired only by the larvae. After a five-day incubation period in the vector, both larvae and adults can transmit the virus, and they retain it for life [Black (24)].

Plant bugs.—*Piesma quadrata* transmits sugarbeet leaf curl virus and *Piesma cinerea* transmits beet savoy disease [Wille (187); Coons *et al.* (43)]. *P. quadrata* can acquire the leaf curl virus as nymph or adult, but only the adult can transmit it.

Chewing insects.—Doolittle & Walker (57) reported on the transmission of cucumber mosaic by *Diabrotica* spp. as well as by aphids. Freitag (64) found that squash mosaic virus and two cucurbit mosaic viruses were transmitted by two species of *Diabrotica* and rarely or not at all by aphids, whereas the western cucumber mosaic virus was transmitted readily by aphids and rarely by *Diabrotica*.

Freitag (65) showed that two species of western cucumber beetles retained the virus of squash mosaic for 20 days and indicated that transmission might be through both regurgitation and fecal contamination. However, he also recovered the virus from the blood of infective beetles.

Turnip yellow mosaic virus is transmitted by the chewing insects *Chorthippus brunneus* (Thunb.), *Forficula auricularia* L., *Leptophyes punctatissima* Bosc., and several species of *Phyllotreta* [Markham & Smith (121)]. The *Phyllotreta* beetles acquired the virus in 1 min., became infective in 24 hr., and retained the virus for 4 days. Transmission was apparently through regurgitation of part of the contents of the foregut. No sucking insects transmitted the virus.

Goss (75) showed that grasshoppers transmitted potato spindle-tuber virus. More recently Walters (177) reported that the grasshopper, *Melanoplus differentialis* (Thos.), transmitted potato virus X, tobacco ring-spot virus, and tobacco mosaic virus. Dale (48) suggested that regurgitation may be less important than contaminated mouth parts in transmission of tobacco ringspot. That *Phenococcus citri* can transmit tobacco mosaic was confirmed by Newton (129). Neither Walters nor Newton showed any bio-

logical relationship between the grasshoppers or mealybugs and the viruses they transmitted [Walters (177); Newton (129)].

Cowpea mosaic virus was transmitted by *Cerotoma ruficornis* (Oliv.), which acquired the virus in 5 min. and retained it for 14 days [Dale (47)]. Fasting did not affect the insect's ability to transmit the virus. No sucking insects transmitted this virus.

Dale (48) suggested that the failure of aphids to transmit viruses causing turnip yellow mosaic, cowpea mosaic, eggplant mosaic, and tobacco ringspot and also potato virus X may be a result of inactivation by some substance that the aphids produce. Occasional transmissions of the squash mosaic or the tobacco mosaic virus might result when small quantities escape inactivation [Freitag (65); Hoggan (84)]. The viruses transmitted by chewing insects may exhibit the ultimate degree of nonpersistence in aphids, but they persist for varying periods in certain biting insects that produce no inactivator [Dale (48)].

Mites.—As early as 1927 Amos *et al.* (3) reported transmission of black currant reversion virus by the mite *Eriophyes ribis* Nalepa. In 1955 Slykhuis (148) demonstrated transmission of wheat streak mosaic virus by *Aceria tulipae* Kiefer, Flock & Wallace (60) the transmission of fig mosaic virus by *Aceria ficus* (Cotte), and Jones *et al.* (100) the transmission of peach mosaic virus by an unidentified eriophyid species.

In experiments by Slykhuis (148) *A. tulipae* transmitted the virus in all active stages, retained the virus for several days and through the molts, but did not pass it through the eggs.

SPECIFICITY OF INSECT VECTORS

In investigations on plant viruses the correct identification of both the viruses and their vectors is important. Day & Bennetts (51) mentioned several virus diseases, for example, beet curly-top in Argentina, Brazil, and the United States, that seem to be closely related on the basis of symptomatology and other properties and yet have different leafhopper vectors. On the basis of vector relationships, recognized by some virus taxonomists to be a better criterion of affinities than symptomatology, it appears that these diseases are caused by at least four viruses [Andrews (4); Black (24)]. Day & Bennetts (51) listed the vectors and nonvectors of 78 plant viruses. These authors cited instances in which an insect had been claimed by one author to transmit a virus and by another to be incapable of transmitting it. They questioned the identity of the virus but not that of the insect. Dickson (52) published the synonymy of aphid vectors of viruses, including the preferred American and European names.

Some mechanically transmitted viruses have a wide variety of vectors. For example, spindle tuber virus is transmitted by both sucking and chewing insects, including aphids, grasshoppers, and beetle larvae and adults [Bawden (9)]. Onion yellow dwarf, transmitted by more than 50 species of aphids, is

an example of group specificity [Tate (169)]. Other examples include Pierce's disease of grape transmitted by 24 xylem-feeding leafhoppers belonging to the Tettigellinae or Cercopidae [Frazier (61); Frazier & Freitag (62); Freitag *et al.* (68); Severin (145)] and phony peach virus by four species of xylem-feeding sharpshooters belonging to the Tettigellinae [Turner (172)]. Marked specialization occurs in the transmission of clover club leaf by *Agallioptis novella* [Black (20)], of curly top of sugar beet by *Eutettix* (= *Circulifer*) *tenellus* [Bonquet & Hartung (27)], and of eastern aster yellows by *Cicadula sexnotata* (= *Macrostes fascifrons*) [Kunkel (108)].

The great diversity of reported vectors of potato leaf roll virus [Day & Bennetts (51)] indicated the need for further investigation of the problem, particularly with reference to the vectors that may have been mixed in cultures. Some of these species were found by other workers to be nonvectors on potato, i.e., *Macrosiphum gei* (Koch) (154), *Myzus solani* (Kltb.) (45), and *Phlaenus spumarius* (= *leucophthalmus*) L. (58). Kassanis (101) stated that insects other than aphids have not been found to act as vectors of leaf roll. McCarthy (123) found *M. persicae* to be the most efficient vector but that *M. solani* and *M. ornatus* Laing could also transmit leaf roll. Neither McCarthy nor K. M. Smith (154) obtained infection with *Macrosiphum solanifolii* on potato, but Kirkpatrick & Ross (103) obtained it with both *M. solanifolii* and *M. solani* when *Physalis floridana* and *P. angulata* were used as source and test plants.

Oman (130) pointed out several errors in identification of vectors of virus diseases and indicated the probable species involved, based on the knowledge of the taxonomy and distribution of the leafhoppers. Had specimens been available to him, more satisfactory identification would have been possible. It is evident that the earlier workers sometimes used mass cultures in their transmission tests and failed to collect and identify all the species involved. At least some recent workers recognized that they had mixed colonies. Leaf crumple of cotton in California was transmitted by adult whiteflies collected in the field from populations that always included a mixture of *Trialeurodes abutilonea* (Hald.) and *Bemisia inconspicua* (Quaint.). Dickson *et al.* (53) were unable to determine whether only one or both species carry this virus. In their report on successful transmission of the virus causing blueberry stunt, Tomlinson *et al.* (171) indicated that they were using a mixture of two species of leafhoppers, *Scaphytopius magdalenis* (Prov.) and *verecundus* (Van D.). Maramorosch (120) tested these species separately and determined that only *S. magdalenis* was responsible. Other cases of mixed colonies have undoubtedly occurred without the worker realizing it [Daniels (50); Jones *et al.* (100)].

The importance of vector specificity as a means of separating viruses and strains of viruses is being recognized. Posnette (133) separated 17 strains of cacao swollen shoot virus carried by 10 species of mealybugs, and Black (18) found distinct vectors for related strains of potato yellow dwarf

virus. However, investigators today are being confronted with so-called physiological races of certain species that are indistinguishable by the morphological characters now known. Examples are the active and inactive races of *Cicadulina mbila* and *Aceratogallia sanguinolenta* (Prov.) [Storey (164); Black (19, 24)].

F. F. Smith (150) pointed out several reports in the literature in which the identity of the vector was indefinite and suggested that some plan be developed for preserving the proved insect vectors of viruses. Unless the investigator used mixed colonies and preserved only a portion of the insects, it would be possible to determine, for instance, whether or not *Philaenus leucophthalmus* transmitted peach yellows, as reported by Manns (113). That *Macrosiphum solanifolii* was the vector used by Allard (1) in transmitting tobacco mosaic can be verified, because specimens from his experiments are located in the Smithsonian collection.

CONFUSING SYMPTOMS CAUSED BY FEEDING INJURIES AND VIRUS INFECTION

Insects and mites that secrete toxic substances into the plant cells or derange the tissue by their feeding induce toxemias with symptoms usually distinctive for the species concerned. Most of these insects are Homoptera or Hemiptera, and the mites, Eriophyidae or Tarsonemidae. The available information on plant injuries caused by insect and mite feeding was reviewed by Carter (40) in 1939. Certain species that cause direct feeding injuries may also be responsible for the transmission of virus diseases. Separation of the two types of injury is seldom easy, but it is necessary for proper diagnosis. We can discuss only a few of the many examples of confusion between plant injury and virus symptoms on economic plants.

Capitophorus fragariae (= *Pentatrachopus fragaefolii*) causes crinkling, splitting, stunting, and discoloration of strawberry leaves and also is an important vector of strawberry crinkle, yellows, and yellow edge viruses [Briton-Jones & Staniland (38); Prentice & Woolcombe (135)].

Myzus solani, reported to be a vector of a virus disease of Easter lilies, was shown later to cause direct feeding injuries that resemble mosaic and rosette transmitted by other aphids but not by *M. solani* [Gadd & Loos (73); Smith & Brierley (152)]. This aphid is at most a poor vector of many virus diseases that are transmitted efficiently by *M. persicae*, which causes no direct feeding injuries at low population levels. However, *M. solani* causes severe chlorotic spotting on tomato after short feeding periods and is also the most efficient vector tested for transmission of tomato aspermy virus [Brierley, Smith & Doolittle (37)].

Various symptoms that have sometimes been attributed to virus infection are now known to result from direct feeding, as by the potato leafhopper, *Empoasca fabae* (Harr.), on its many hosts; or from insect toxins, as by the potato psyllid, *Paratrioza cockerelli* (Sulc.), on tomato and potato. Husain (91) very likely confused symptoms of direct feeding injury on cotton by

Empoasca devastans Dist. with those of cotton leaf curl. Likewise symptoms on cotton resulting from infestations by *A. gossypii* have doubtless been confused with symptoms of cotton leaf crumple, which is transmitted by whiteflies [Dickson *et al.* (53)].

In unpublished experiments by the present authors, *Myzus dianthi* (Schrank) on greenhouse carnation caused symptoms that resembled carnation streak. *M. persicae* died if confined to carnation and caused no chlorotic injury. It was an efficient vector of carnation mosaic virus, but *M. dianthi* did not transmit it. Although living specimens of *M. dianthi* are readily distinguished from those of *M. persicae*, preserved specimens are not. In an earlier report by Jones (98), the direct feeding injury caused by *M. dianthi* was confused with symptoms of carnation streak, which has no known vector, and this aphid, which was mistakenly called *M. persicae*, failed to transmit carnation mosaic.

Morphological characters of preserved specimens are of value in differentiating the toxemia-inducing species *Amphorophora rubitoxica* Knowlton, which is a nonvector of raspberry viruses, from *Amphorophora rubi* (Kalt.), which does not induce chlorotic spots and yet is an efficient vector of leaf mottle and yellow mosaic viruses on raspberry [Wilcox & Smith (186); Stace-Smith (158)]. These two raspberry aphids are indistinguishable in the field.

Eriophyid and tarsonemid mites are noted for their direct feeding injuries. Also on record are three cases of plant-virus transmission by eriophyid mites. The big bud disease of black currant is evidently caused by feeding of *Eriophyes ribis* Nal., and the virus of the reversion disease on the same host is transmitted by this mite [Lees (111)]. *Aceria tulipae* causes feeding injury on wheat and also transmits wheat streak mosaic virus [Slykhuus (148)]. The feeding injury by *Hemitarsonemus latus* (Banks) was described as a new virus disease of potato but later corrected by the same author [Reddick (138)].

CONTROL OF VECTOR-BORNE VIRUS DISEASES

In the control of vector-borne virus diseases attempts are made to eliminate reservoirs of the virus and to avoid, exclude, or destroy the vectors. Potato-seed stocks are commonly produced in climates too cool, too moist, or too windy for extensive buildup of the aphid vectors of important potato viruses. China asters are often grown in cloth-covered enclosures to exclude the leafhopper vector of the aster yellows virus [Jones & Riker (99)]. Greenhouses also exclude most leafhoppers, so that hopper-borne viruses are rarely troublesome. The same species of thrips and aphids are controlled more effectively in greenhouses than in the open field [Gardner & Michelbacher (74)].

As Heinze (80) pointed out, the control of disease vectors with potent organic insecticides, particularly the systemics, was undertaken with high

hopes. However, it is far more difficult to control vectors than insects that cause direct injury. The latter are controlled when their numbers are reduced to a point where damage is negligible. On the other hand, to control a vector the insecticide must be applied early in the season before there is any infestation, and it should kill each new arrival before it feeds. Experiments have shown that the best of the new insecticides fail to kill aphids fast enough to prevent transmission of sugar-beet yellows virus or of potato leaf roll virus [Bawden (10); Roland (140); Heinze (80)]. In the writers' unpublished tests with *A. gossypii* and the persistent lily rosette virus, infections in plants treated with heavy dosages of demeton or schradan were about half those in untreated plants. Viruliferous aphids infected plants within 1 hour's feeding, whereas they succumbed to the systemic poisons only after 12 to 16 hr. Potato leaf roll virus can also be acquired from demeton-treated plants and beet yellows virus from schradan-treated plants and transmitted to untreated plants. Although aphid transmissions of persistent viruses to treated plants tend to be fewer than to untreated plants, the nonpersistent viruses are less affected by treatment. Heinze (80) noted that aphids placed on plants sprayed the preceding day with parathion or demeton were stimulated to unusual activity and made more feeding punctures and more transmissions of nonpersistent viruses than those on untreated plants. On plants sprayed four days previously they were sluggish and made fewer and longer feeds. Hull & Gates (90) noted similar excitation of *M. persicae* and increased spread of beet yellows following applications of DDT and chlordane to sugar beets.

In field tests demeton spraying reduced the leaf roll in the harvested potato crop more than it reduced the nonpersistent mosaic viruses [Heinze (80)]. Experimental plots sprayed with various aphidicides remained green longer than unsprayed plots and attracted late migrant aphids [Muenster & Murback (126); Bronson *et al.* (39)]. In Maine the potato growers have sprayed their crops with DDT or other aphidicides since 1945, primarily to destroy insects and to increase yield [Shands & Simpson (147)]. This practice has reduced aphid populations, especially the summer alates of *M. persicae*, to such low levels that spread of potato leaf roll has declined. The quality of potatoes has improved so much that the tolerance for leaf roll in certified potatoes has been lowered from 2 to 1 per cent. Similarly, spraying of sugar beets with demeton over large areas in Germany has resulted in a 20 per cent increase in yield of beets and a 28 per cent increase in sugar content [Dame & Goossen (49)].

From 50 to 90 per cent reduction in aster yellows infection in lettuce and carrot has been reported in New Jersey [Pepper & Haeuseler (132)], New York [Ashdown & Watkins (5); Hervey & Schroeder (81)], and Maryland following repeated treatments with DDT. In Maryland applications at five-day intervals were necessary to destroy reinvading leafhoppers [F. F. Smith (unpublished data)]. Spraying weed borders with parathion or malathion

was found to be commercially practicable for controlling aster yellows in Michigan lettuce fields [Hoffman (83)].

To protect California sugar beets, tomatoes, and other crops from infection with curly-top virus, the overwintering food plants of *Circulifer tenellus* in the nearby foothills have been sprayed annually with various insecticides since 1931. Although the results are difficult to evaluate, no serious general outbreaks of curly top have occurred since the program was inaugurated [Freitag (66)].

Spraying peach orchards with DDT in June led to a reduction of *Macropis trimaculata* (Fitch) and the peach yellows virus that it transmits. This vector has but one generation per year and is active on peach only from May to August [Turner (173)]. Similarly, spraying with parathion, TEPP, or lindane checked the spread of strawberry yellows virus, transmitted by *Capitophorus* (= *Pentaltrichopus*) *fragaefolii*; both vector and virus reservoir occur within the strawberry fields [Breakey & Campbell (31)].

Spraying with insecticides had no measurable effect on infection of cantaloups by the nonpersistent cantaloup mosaic virus transmitted by migrant aphids from beet fields [Dickson *et al.* (54)]. Jenkinson & Glynne Jones (94) failed to protect cauliflower sprayed with schradan against infection by cauliflower mosaic transmitted by *Brevicoryne brassicae* (L.). Evans (59) sprayed peanuts with schradan to control the peanut rosette virus transmitted by *Aphis craccivora* Koch. Primary infections were not prevented, but no secondary infections by apterae occurred. Evans considers that elimination of volunteer peanuts between crop periods, together with vector control, will afford practical control of the disease. Similarly, Swenson *et al.* (166) obtained 50 to 67 per cent reduction of enation pea mosaic in peas with parathion and demeton sprays against the vector, *Macrosiphum pisi*, migrating from alfalfa and clover. Not all the primary infections were prevented, but secondary spread was almost eliminated.

The usual insecticide sprays failed to control the mealybug vectors of cacao swollen shoot virus, because of the protective tents constructed over the mealybug colonies by the ant attendants [Posnette (134)]. However, Hanna *et al.* (78) found the systemic insecticide bis(dimethylamino)fluorophosphine oxide highly toxic to mealybugs on cacao. When applied to the soil or to the bark of cacao trees, it reduced mealybug populations by 99 per cent. Eight treatments at six-week intervals protected cacao for a year, which was sufficient time for expression of any latent infections. The treatment has not been adopted commercially, but promises to afford practical control of swollen shoot.

Thus field control of plant virus diseases through vector control is more likely to succeed when virus reservoirs are chiefly within the crop, for there is then an opportunity to destroy the vector before it leaves the source plant. Also, when very large areas can be sprayed, the vectors are likely to be destroyed before they penetrate deeply into the crop. Spraying smaller

border areas is helpful in checking invasion of strong-flying vectors such as the aster leafhopper.

EFFECT OF CHANGE IN AGRICULTURE ON SPREAD OF VIRUS DISEASES

The discovery of more than 200 new viruses since 1925 suggests that some of them are new [Bennett (15)]. However, viruses do not arise anew any more than plants or animals do, although new strain variants arise from old stocks comparable to new plant mutations or sports [Bennett (15); Brierley (33)]. Therefore, it is more likely that most of the so-called new viruses are merely newly recognized or new to a given crop or locality.

Some of our common viruses attained worldwide distribution through commerce in plant parts. Examples are spotted wilt in dahlia tubers, several viruses in gladiolus corms, the viruses in tomato and potato, and tobacco mosaic in tobacco products [Holmes (88)]. With modern air transportation, hitch-hiking insects could readily carry persistent viruses from one continent to another. Even the mealybug vectors of swollen shoot, which retain the virus for 36 hr. in the absence of food, could be carried between continents [Posnette (133)].

Cultivated crops introduced into new areas may become infected with viruses from native vegetation transmitted by their vectors. The virus causing yellow wilt of sugar beet, endemic in Argentina and transmitted by the endemic *Alanus exitiosus* Beamer, has destroyed the sugar-beet industry introduced into that country [Bennett (15)]. Cacao introduced into West Africa became infected with virus from native trees by native mealybug vectors, with consequent rapid spread of the swollen shoot disease until the entire cacao-growing industry in Africa was threatened [Posnette (134); Hanna *et al.* (78)]. The extension of stone-fruit culture into new areas of North America during the last 20 years has been followed by the recognition of 38 new virus diseases. Some of these diseases have been found in native plants. Another example is the occurrence of Pierce's disease of grape in many wild hosts and transmission of the virus by several species of leafhoppers that never attack grape [Bennett (15); Turner (173)].

Sometimes a highly efficient vector of an indigenous virus may cause serious disease outbreaks when introduced into a new area. For example, the beet leafhopper may have been introduced into western North America by early settlers from its native Mediterranean area. The curly-top virus is apparently a native of the Western Hemisphere and possibly carried by agallian leafhoppers [Freitag *et al.* (69)].

A virus introduced into a new area will not necessarily damage crops under all conditions. Although the spotted wilt virus is probably present in most dahlia plantings throughout the United States and the thrips vectors are also present at certain seasons, it is only in localized areas, such as around San Francisco Bay, that the disease spreads to other agricultural crops. Tomato aspermy virus has not spread to tomato and other crop plants in this country, though introduced in chrysanthemum into at least six States

and though the two crops are commonly grown together in gardens. Lack of spread may be attributable to its low concentration in chrysanthemum plus the fact that its several aphid vectors colonize only chrysanthemum or rarely feed on this host [Brierley *et al.* (37); Hollings (86)].

With the demonstration that certain viruses are common to both plants and insects and reproduce in both, Bawden pointed out that viruses may have originated in hosts remote from those in which they are currently most important and most studied [Black (22)]. It is possible that sources of some plant viruses may be the insect pests that are their symptomless vectors.

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NONBITING FLIES AND DISEASE¹

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The nonbiting flies are probably responsible for the transmission of the causative agents of many more cases of enteric bacterial infections, of all types, than are biting flies, including mosquitoes. Certainly this is true in the United States where infection rates, if not disease, are low in comparison to those of the rest of the world. From surveys conducted in New Mexico, Georgia, Puerto Rico, and New York City, Watt & Hardy (1) showed that *Shigella* infections predominated, their studies indicating total reported or estimated annual morbidity rates of 48 per cent in New Mexico, 20 per cent in Georgia, 60 per cent in Puerto Rico, and "markedly less" in New York City. They also found that

... for every known infection (manifest source) there are numerous unrecognized infections (hidden sources). In the light of these findings it is not surprising that endemic diarrheal diseases commonly appear to be scattered sporadic cases. These seemingly unrelated infections may arise from a single source or be joined by a series of undetected infections. This knowledge is essential for the interpretation of the epidemiology of the acute diarrheal diseases.

In many tropical and subtropical areas devoid of modern sanitation, the principal factor influencing the spread of disease agents by flies and other means is the degree to which the etiologic agents themselves are present. This is also true in many marginal areas with inadequate sanitation and may likewise occur in areas of normally high standards during temporary disruptions of sanitational facilities following disaster. Atomic warfare, for example, would create such disruption on an unprecedented scale.

HISTORICAL REVIEW

In Chapters XI, XII, and XIII of his recent (1951) book, West (2) has reviewed the literature extant through 1949 on "Flies and Human Disease," "Public Health Relations," and "Myiasis," respectively. In most instances, he did not attempt a critical review of the early experimental work dealing with the passage of disease organisms through flies. Since this paper is concerned specifically with an evaluative appraisal of studies on nonbiting flies and disease, the authors are following West's example in omitting critical

¹ The literature review incorporated in this paper terminated June 1, 1955.

evaluation of the early work as it is believed that this would involve too much speculation to serve any real purpose.

STUDIES PRIOR TO WORLD WAR II

Much of the work preceding World War II on the passage of organisms through flies was performed in England during the few years immediately before World War I. Not only were bacteriological techniques and taxonomy relatively undeveloped at that time, but little was recognized of the complexity of the problem involved. Thus controls on the experiments were generally inadequate, and the organisms involved are now impossible to identify in modern terms. This is no reflection upon the workers of the period; the investigations during the first two decades of the century by Graham-Smith, Austen, Faichnie, Nicoll, Bacot and Hewitt, to mention only a few, were truly pioneering, conceived and executed with ability quite as comparable to the techniques and knowledge of the day as are our best modern efforts. Several papers by each of these men are mentioned by West (2).

By way of comparison, the earliest work on the ability of flies to serve as mechanical vectors is probably as significant today as when it was done. While the earliest accusations against flies were speculative, several classic experiments were performed around the turn of the century and are well documented in books by West (2), Riley & Johannsen (3), Herms (4), and Matheson (5). The primary significance of this early work, as well as of all later studies, lies in the fact that under certain optimum conditions flies can be as effective in spreading infection as are fingers, dirty eating utensils, and contaminated food. The habits of many of the flies, particularly of the house fly, *Musca domestica* L., enhance the acquisition and spread of disease organisms found in feces and other excreta. While the exteriors of most flies are well adapted for transporting contaminants from one place to another, there is no evidence of any specific physiological advantage of flies over other insect orders, such as many Hymenoptera, for example. In short, the habits of flies make them the disease-vectoring threat they are, and those of the house fly probably qualify it as the chief malefactor.

STUDIES SINCE WORLD WAR II

During World War II large-scale military operations in tropical and subtropical areas revived consideration of flies as enteric disease vectors, and at least two episodes of enteric infections were so definitely associated with fly populations as to qualify them as being probably fly-borne. Two 1944 papers describe the experiences of Stewart (6) in North Africa and of Kuhns & Anderson (7) in an unnamed southern army camp in associating epidemics of bacillary dysentery with fly abundance. Both papers correlated certain fly control operations with presumed effects upon the courses of the epidemics. These studies are referred to by West (2), but he does not point out their presumptive nature. The work of Watt & Lindsay (8) is also mentioned by

West (2), but without comment other than that it was a controlled study; actually, it was the first such controlled study of the role of nonbiting flies in any human disease.

In spite of evidence demonstrating the ability of the various nonbiting flies to carry disease agents, and of observations showing the association of an abundance of flies with increased prevalence rates of various diseases, chiefly enteric, there existed prior to 1946 no experimentally derived proof of the true role of flies in the spread of any pathogen. West (2) points out the circumstantial evidence involved in fly vectoring and reports the pre-World War II statements of several workers to the effect that flies were probably only supplementary to a more common form of transmission in most diseases.

Poliomyelitis.—In considering flies and poliomyelitis the reader is referred to West's discussion of the "Contributions of the Past Decade" (2, pp. 283-86) summarizing the research on fly transmission of poliomyelitis virus and commenting upon certain aspects of it. From the work cited one is left with few doubts that flies may harbor poliomyelitis virus for varying lengths of time and, under the proper conditions, transmit these agents either by external contamination or internal passage following ingestion.

The finding of polio virus in human feces by Trask *et al.* (9, 10) emphasized the possibility of nonbiting flies being involved in the transmission of the disease. The recovery of virus from sewage by Paul *et al.* (11), Paul & Trask (12), Toomey (13), and Kling *et al.* (14), and the finding of antibodies against mouse-adapted Lansing strain virus in animal sera (presumably indicating that infective animal feces sometimes were found) by Hammon (15) and Gordon (16), demonstrated the ready availability of virus to flies from commonly occurring sources during epidemics. These disclosures, occurring in the late 1930's and early 1940's, were followed by several studies on the natural occurrence and laboratory survival of poliomyelitis virus in several species of flies and, through 1945, are well summarized by Francis *et al.* (17). Also at about this time Ward *et al.* (18) fed fly-contaminated food to two chimpanzees, resulting in subclinical poliomyelitis infections in both animals. Virus persisted in their stools for one to two months and caused typical cord lesions in rhesus monkeys upon passage.

These findings induced many authorities in the field to incline toward the so-called "fly theory" of epidemic transmission of poliomyelitis. Even those not so inclined readily admitted the persuasiveness of the data and were probably most influential in making the decision to conduct an early test of the theory by means of fly control. Until the miracle of DDT late in World War II, no definitive studies of the effects of fly control on any disease were feasible as a result of the difficulties in achieving and maintaining the control of flies.

Among the recognized obstacles facing such a test were the following: (a) All the significant epidemiological studies of poliomyelitis showed a definite pattern of personal contact between cases; (b) The incubation period

of the disease, added to the time necessary for mobilizing and for achieving fly control with DDT after the recognition of an incipient poliomyelitis epidemic, probably would make the control of flies an achievement that followed the peak of actual but as yet inapparent infections; (c) There are many obvious objections to using untreated comparison areas within the environs of the experimental fly control zone in view of the ability of flies to migrate and invalidate such areas as controls; and similar areas some distance away rarely are truly comparable; (d) For the first time DDT was to be used under civilian rather than military conditions, involving voluntary agreements and widespread but individual acceptance of the measures to be used; and (e) The exact measures to be used had not been established and had to be developed.

Actually these conditions were so overwhelming that, in retrospect, it is now easy to understand the inconclusive results of these experiments (19), conducted in Paterson, New Jersey and in Rockford, Illinois. In fact only truly negative results, or the occurrence of a full-blown epidemic in spite of fly control, would have been of much significance even had the element of time been overcome. Entirely through chance such observations, described by Paffenbarger & Watt (20), were possible three years later. A poliomyelitis epidemic occurred in an area where fly control was being carried on to study its effect upon enteric infections (8). In spite of the maintenance of high degrees of fly control in four of nine towns in Hidalgo County, Texas, the rates of paralytic cases were nearly identical in towns with and without fly control. In addition, epidemiological investigations revealed that the attack rate per 1000 was 12 times as high among individuals with histories of contact with recognized cases of poliomyelitis as in individuals with no histories of contact, regardless of fly treatment status of the area of residence.

To conclude the review of studies of the effects of fly control upon poliomyelitis, two other groups of individual studies may be mentioned. The first of these, during 1947, centered primarily in Wilmington, Delaware, and was inconclusive as to epidemiological results. The second consisted of studies begun in 1948 in Topeka, Kansas; Troy, New York; Muskegon, Michigan; Charleston, West Virginia; and Phoenix, Arizona. Studies in the first three areas were terminated in 1950 and in the latter two in 1953. The longer duration of these studies permitted utilization of prophylactic sanitation as well as the use of chemicals in the control program.

Coxsackie virus.—One interesting finding of the Wilmington study was that the fecal specimens from many of the clinically diagnosed poliomyelitis cases later yielded only Coxsackie virus, as reported by Dalldorf *et al.* (21) and Dalldorf (22). Several publications emanated from the second group of studies, but probably of greatest significance is the report by Melnick *et al.* (23) that both Coxsackie and poliomyelitis viruses were isolated from various species of flies collected in the various cities.

Bacillary dysentery.—Any disease that is as equally capable of being fly-vectored as poliomyelitis, and is also highly prevalent and endemic in a study

population, would lend itself better than poliomyelitis to a study of the role of flies in transmitting infections under normal living conditions. The disease should be so prevalent that a reduction of 50 per cent in rate would be readily detectable in a sample of feasible size. A disease with such qualifications is found in some parts of the southern United States and is caused by the complex of etiologic agents producing the types of enteritis generally known as "bacillary dysentery." In 1945 the late Joseph W. Mountin, then Assistant Surgeon General of the Public Health Service, and Albert V. Hardy, then of the National Institutes of Health, Public Health Service (now Director of Laboratories for the Florida State Board of Health) recognized this potential for establishing the role of flies in human disease. They initiated the study conducted by Watt & Lindsay (8) in Hidalgo County, Texas in 1946 to 1948. Hidalgo County is located near the mouth of the Rio Grande River and has a semiarid and subtropical climate. Its entire fly problem arises from inadequate disposal of wastes, chiefly from large vegetable and citrus packing and canning industries. Through co-operation with the Texas State Health Department, fly control was achieved with DDT spraying in five out of nine similar towns. It resulted in substantial reductions in (a) *Shigella* infections, as determined by rectal swab cultures in representative groups of children under 10 years of age, (b) reported attacks of diarrheal disease, and (c) reported deaths of children under two years of age. Fly control operations were routinely established early in 1946, a few weeks in advance of the establishment of direct epidemiological measurements. Thus differentials between reported illnesses and actual infections were evident when first measured, although reported deaths of infants under two years of age prior to fly control were essentially at the same rate in the two groups of towns. In September, 1947, after nearly 20 months of fly control in five of the nine study towns, the treatment order was reversed, and the four previously untreated towns were subjected to fly control measures with DDT. This change in schedule coincided with the normal seasonal increase in flies in the area and within a few days the Scudder fly grill indices (24) for the previously treated towns had risen sharply. This was followed a few weeks later by a comparable condition in the rates of cultured infections of *Shigella* and of reported diarrheal disease. Concurrently with these rising trends, fly indices for the currently treated towns abruptly declined. This reversal in trends, coinciding with the change in fly controlled areas, decisively eliminated the possibility of chance and demonstrated the role of flies in vectoring a human disease in this study area and under the conditions involved.

The rapid reversal in fly densities, and subsequently in diarrheal disease and *Shigella* infections, was enhanced by the fact that the house fly population had attained a high degree of resistance to DDT. Control in the initial five towns during the last four months prior to September, 1947 had been maintained with great difficulty and relatively enormous quantities of DDT. The rise in fly populations was due almost entirely to a single species, the ubiquitous house fly.

The experience in Hidalgo County, an area of high diarrheal disease morbidity, was duplicated in 1949 and 1950 in Thomas County, Georgia, an area of moderate diarrheal disease morbidity. This work was reported by Lindsay, Stewart & Watt (25). In addition to the differences in morbidity and mortality found between south Texas and southwest Georgia, there were far greater differences in climate, agricultural practices, human population density, and primary sources of fly breeding. Except for the seasonal cannery wastes in Hidalgo County, the Georgia area provided an environment much more suitable for fly breeding, and a similar reduction in diarrheal disease morbidity was found to follow fly control. Mortality rates, however, were too low to be properly evaluated. In this instance an epidemiological evaluation of the entire area prior to fly control was made and demonstrated the equal comparability of both cultured *Shigella* infections and reported diarrheal disease in both treated and untreated towns. In Thomas County there had been large-scale agricultural use of DDT and other chlorinated hydrocarbons with the inevitable result that DDT was only effective against the predominant house fly during the initial year of the study. When dieldrin and chlordane were substituted, the degree of fly control obtained was excellent, but resistance in the house fly population was evident within the span of two generations. Thus by September 1950, after only 14 months of chemical control of flies, the level of fly control that could be maintained was unsatisfactory and was reflected by sharp increases in the diarrheal disease morbidity and infection rates found in the human populations of the treated towns. There is no doubt but that the house fly was the primary fly vector in this study also, but this does not imply that other species present in similar concentrations could not have performed with the same vectoring efficiency. In this instance the attempted switching of treated and untreated towns was unsuccessful as a result of the rapid acquisition of a high degree of house fly resistance to all the insecticides used.

Many studies on shigellosis have been carried out by the United States Naval Medical Research Unit Number 3, Cairo, Egypt. Higgins & Floyd (26), of this unit, reported studies on the distribution, chemotherapy, and immunological aspects of shigellosis in rural towns in Egypt. Although they did not specifically correlate these data with measurements of fly populations, the following quotation is believed to be pertinent to this discussion:

Flies. The housefly, *Musca domestica*, is a prominent feature of Egyptian village life, being prevalent in fantastic numbers from May to October, with some decrease in breeding during the hotter months of July and August. The transmission of fly-borne disease may be uniquely affected by the habits of one of the locally prevalent species, *M. domestica sorbens*, whose preference for breeding in human feces, and whose predilection for feeding on human secretions, described by Peffly (1953), leads to the startling appearance of children with eyes, nostrils, and mouth ringed by flies. Children and mothers accept them, and make little or no attempt to brush them away, since they will be replaced by the same or other flies. In this village, the fly prevalence was effectively controlled by insecticides in 1950, and infant mortality dropped from

227/1000 live births in 1949 to 115/1000 in 1950. In 1951, however, the fly population became resistant to chlordane and DDT, and returned to its former level. Infant mortality also again resumed its previous level. A large part of the salvage of infant life during the period of fly control was attributed to control of fly-borne disease (Weir *et al.*, 1952).

In the Georgia study, as in the preceding Texas study, a notable and surprising failure to demonstrate any role of flies in the transmission of salmonellosis bacteria is still unexplained. Considering the extremely wide distribution of *Salmonella* infections in domestic animals in both areas, it seemed probable that flies might play a part in carrying the agents of such infections from the feces of their usual animal hosts to their unusual human hosts. No apparent effect of fly control upon *Salmonella* prevalence rates was detectable, although the low initial prevalence of such infections may have masked any real difference. In two reports, however, Watt & DeCapito (27) and Stewart & DeCapito (28) presented good cases for direct contact between the animal and human hosts. The relatively low prevalence of *Salmonella* infections in man in these two areas demonstrates either the low vectoring potential of flies or the unnatural host qualifications of man, or both. Milner & Shaffer (29) pointed out that under experimental conditions *Salmonella* infections are surprisingly hard to establish intentionally in laboratory animals considering the apparent ease with which incidental infections developed. They found day-old chicks rather uniformly susceptible and have used them to establish certain baselines in the study of salmonellosis.

McCullough & Eisele (30, 31, 32) fed human volunteers various dosages of *Salmonella*. McCullough has indicated to the authors that the dosages shown necessary for establishment of infection in man are probably greater than one would expect a fly to carry externally or to deposit on human food through defecation or regurgitation at any one time. This low-dosage transmission may well be the explanation for poor vector efficiency of flies going from animal excreta to man's food, particularly as man is not an easily infected host. The coprophagous habits of animals indicate that flies are unnecessary for vectoring of infections. Man's infections with *Salmonella* are believed to stem usually from infected eggs, meats, or meat products in which natural infections have had opportunities to incubate and reproduce. Such situations are usually found in instances of large outbreaks of salmonellosis.

Infectious conjunctivitis.—Other studies of nonbiting flies associated with human disease have involved the chloropid eye gnats (*Hippelates* spp.) and their seasonal abundance coincidental with "pink eye" or infectious conjunctivitis. Bengston (33) studied the disease in southwest Georgia in 1933 to determine the etiologic agent, and her presentation of circumstantial evidence for *Hippelates* vectoring reflects their classically ascribed role. Davis & Pittman (34, 35) and Davis & Hinds (36) reported upon the etiologic agent and its distribution from studies conducted in Hidalgo County,

Texas, and Thomas County, Georgia. Of particular importance was their finding that the Koch-Weeks bacillus, now identified as *Hemophilus aegyptius*, and *H. influenzae* were present in a significant number of cultures from "sore eyes." Davis (37) has reported that *Hemophilus* infections have persisted in asymptomatic individuals in Thomas County for periods of several months; the significance of this observation is in relation to the source of infection in each year's spring or early summer increase in pink eye. Dow (38) demonstrated the feasibility of *Hemophilus* transmission by *Hippelates*, particularly *H. pusio* which is more attracted to man and animals than is *H. bishoppi*.

Continuing studies by Tinkham (39), in the famous gnat-frequented Coachella Valley in southern California, have shown markedly positive correlations between the numbers of *Hippelates collusor*, the dominant species present, and all school absenteeism. Great progress in the control of *Hippelates* breeding has been made through both the use of chemical insecticides and cultural practices in date groves. These reductions in gnats have been paralleled by decreases in school absenteeism proportionally in all grades. Earlier work had demonstrated that "sore eyes" were considered the primary cause of school absenteeism in the area. Although Tinkham's data are inferential and do not rule out other and perhaps unrecognized circumstances that might have affected the prevalence of "sore eyes," they represent the best evidence we have on the role of the eye gnat in vectoring *Hemophilus* organisms.

Muirhead-Thomson (40) reported in 1954 that *Siphunculina funicola* coincides in seasonal incidence with Maga sore and epidemic conjunctivitis in Assam India.

Trachoma.—Although the etiology of trachoma is unclear, the recent report of Siniscal (41) characterized trachoma as a family disease not highly contagious to others and indicated poor personal hygiene in crowded insanitary areas as basic to transmission. If his conclusions are sound, flies would seem very unimportant as carriers during ordinary levels of the disease; quite contrary to the widely held belief that flies transmit a complex of etiologic agents and exudate which causes the disease.

Yaws.—Studies of yaws in western Samoa by Satchell & Harrison (42) have pointed most convincingly to the transmission of the spirochaetes of this disease in part by wound-feeding flies. They considered the prerequisites for yaws transmission by *Musca domestica* and *Musca sorbens* to be amply demonstrated by their work. These two fly species were found to make up respectively 52.3 per cent and 47 per cent of 1,037 wound-feeding Diptera collected in Samoa. In addition to their predilection for wounds, these flies were shown capable of picking up spirochaetes from yaws lesions and retaining the motile spirochaetes in their crops for periods up to 2 hr.

Other human diseases.—The causative agents of some 30 diseases in all have been associated with or demonstrated by laboratory techniques to be capable of transmission by nonbiting flies. Circumstances recounted by

various authors are very convincing in the incrimination of flies, particularly when we are not reminded of the multitude of other modes of spread that occur simultaneously with the flies. Without indicating the degree of importance, it may be said that flies constitute one mode of transmission for the agents of such diseases as cholera, and various protozoan and helminth infections. West (2) has adequately reviewed the studies on these diseases through 1949, and subsequent studies add little to the basic knowledge concerning their transmission.

Animal diseases.—Again the etiologic agent of almost any infectious disease of animals is probably capable of being vectored by nonbiting flies under favorable circumstances, and many candidate diseases are reviewed by West (2). Because the feeding habits of most domestic animals preclude effective sanitation of their food, it is highly doubtful that many of these diseases depend to any major extent upon vectoring by nonbiting flies. Correlations of increased prevalence of such diseases as streptococcal mastitis of cattle with increased fly abundance have been made (43, 44, 45), but increased prevalence in the virtual absence of flies is also found (46). Climatic conditions, particularly temperature ranges favorable to fly multiplication, frequently benefit the survival of various pathogens outside the host.

One infectious agent that would ordinarily be considered as being transmitted in part by flies is the virus of the psittacosis-lymphogranuloma group described by York & Baker (47) in 1951 and designated *Miyagawanella bovis*. This virus is normally present in cattle herds throughout the area around Ithaca, New York. Under certain conditions it produces either symptomatic disease or lack of weight gain in young animals. For the past five years, according to Baker (48), a herd at the Virus Research Laboratory of Cornell University has been maintained free of this virus by means of double-fence isolation and scrupulous sanitation by handlers. The disease-free herd is located less than 300 yards from numerous infected animals. No measures have been taken to prevent the passage of flies between herds, yet the isolated herd has remained free of the virus. Since this virus is readily recoverable from feces and must certainly be ingested or carried on the exterior of flies to the food of the virus-free herd, it appears that this constitutes a well authenticated instance of lack of vectoring ability of flies in the face of overwhelming probability.

In addition to the literature on myiasis cited by West (2), those interested in the subject should review Roberts' book on *Insects Affecting Livestock* (49). This author gave losses attributable to the sheep blow fly at annual figures of 860,000 pounds sterling for Great Britain and no less than four million pounds sterling for Australia. Most interesting is the development of nonsusceptible or "plain-breeched" sheep, advocated by certain investigators as the most effective means of control. The surficial clipping of breech folds (Mules' Operation) can be used on present breeds of sheep to reduce the "wrinkly breeches" condition so conducive to "crutch strike," the most common of sheep myiasis.

Zumpt published a comprehensive monograph on *Myiasis in Man and Animals in Africa* (50) with extensive tables and bibliography.

Most novel of recent control efforts have been those of the U. S. Department of Agriculture, who announced the eradication of the primary screw-worm fly, *Callitroga hominivorax*, from the island of Curacao (51). This was the successful culmination in the field of techniques developed for rearing, radiation-sterilization, and release of male flies by Bushland & Hopkins (52) and Baumhover *et al.* (53). Properly timed release of an overabundance of sterile males for mating with wild females resulted in no progeny, since the females mate but once. The success of this procedure, based on knowledge of fly habits and ecology, is certainly an achievement worthy of commendation in an age when the insecticidal approach tends to rule.

ECOLOGY OF FLIES AND DISEASE

The foregoing accounts of the demonstrated role of flies in vectoring shigellosis under certain conditions and the lack of such demonstration for other infectious diseases are admittedly confusing; nor is the end to the confusion in sight. The recovery of the various pathogens from in or on the various species of flies studied have indicated that the nonbiting flies are possessed of variable vectoring potentials. The specific tests of these potentials have, for the most part, been encouraging to the control of disease in that the survival rate of the test organisms is usually very low. A notable exception is the work reported by Hawley *et al.* (54) purporting to show the multiplication of pathogenic *Shigella* and *Salmonella* organisms during passage through the house fly. The technique in handling the passed feces of the flies that had been fed various calculated doses of organisms has been shown by Haines (55) to allow for significant multiplication in the receiving saline prior to plating, making questionable the conclusions of Hawley and his co-workers that multiplication had occurred within the fly. Haines used newly isolated strains of pathogens and attempted to simulate natural foods and conditions, whereas Hawley *et al.* used laboratory-attenuated strains of pathogens and fed their flies on a highly artificial diet throughout the experiment. The laboratory-adapted cultures of pathogens may well have multiplied within the fly in a manner differing from that of the organisms more recently obtained from human infection. Although, as reported by Melnick & Penner (56), the pathogens recently isolated from human infections are more invasive, they also probably are subjected to more adverse factors than the more inert, attenuated forms. Ingram (57), in making quantitative studies of fly-organism relationships, used a streptomycin resistant strain of *Escherichia coli* and found that food ingested by the fly can influence retention of the organism within the fly; that an interference or competition phenomenon can exist between the introduced organisms and those already present; and that ingested food may alter this latter relationship.

Many other variable factors are also recognized as possible causes of the

diverse results obtained by different workers. Desiccation, time away from nutrients, competition with other organisms, and the effect of bacteriophages are expected to exact a staggering toll of the organisms carried on the exterior of flies. Haines' (55) and Ingram's (57) studies indicate that conditions favorable to the internal passage of viable pathogens are perhaps more difficult to attain than those favoring the vectoring of such pathogens by external contamination.

AREAS WHERE RESEARCH IS NEEDED

Certainly the diverse findings shown above indicate the need for systematic studies of the conditions that promote or inhibit the survival, multiplication, and passage of various microorganisms. Such studies would be of medical as well as of entomological importance, since the knowledge of *in vivo* ecology of pathogens in the fly would add greatly to an understanding of the way in which these pathogens produce disease. Such studies also could be of great importance to epidemiology. The finding by Watt & Hardy (1) that *Shigella* infections greatly exceeded symptomatic shigellosis, with the implication that the disease is attributable at least in part to the physiologic response of the host, was of great importance in explaining the apparently erratic distribution of diarrheal disease often found. Discovery of factors known to limit or promote viability of infections capable of fly vectoring would add similarly to the knowledge of the epidemiology of resultant diseases.

The systematic approach recommended would, of necessity, incorporate much that is tedious and nonspectacular. To be significant it would have to first establish optimum baselines for pathogen survival and then determine the factors inhibiting or enhancing the survival of organisms in flies under conditions to be found in the field. From the information now at hand it is quite apparent that the interrelationships between two or more types of microorganisms, whether or not either or both are pathogenic to man, are as important as the interrelationships between the flies and the organisms. Haines (55), for example, found high mortality in young flies fed broth containing high concentrations of *Shigella*. The relationship of diet to survival of both microorganisms and flies is similarly complex, involving not only the enzyme systems of the host but also those of the ingested microorganisms, in addition to the nutrient components of the diet.

The need for basic research is evident if we are to add to our understanding of the attraction of flies to baits, natural foods, breeding media, and hosts (for those which are myiasis-producers). Eventually basic research may be valuable in developing repellents and even oviposition deterrents for the protection of exposed organic wastes.

Basic studies of the chemoreceptive and sensory organs of blow flies have been the subject of many papers discussed and cited by Dethier in his *Chemical Insect Attractants and Repellents* (58), his chapter on chemoreception in Roeder's *Insect Physiology* (59), and in the review by Dethier & Chadwick

(60). The work of Lewis (61) on the histology of fly tarsi is representative of the most recent trend toward more basic investigations.

Barnhart & Chadwick (62) discovered a "fly factor" in bait attractiveness which seemed to be an odor left by the flies themselves on bait they had visited. Further analysis of the fly factor odor may contribute toward the formulation of more attractive baits for all species or in the design of "species-specific" baits. Chemicals of some specificity may be illustrated by the *iso-valeraldehyde* which Dethier and co-workers (63) found very attractive to *Musca* and *Ophyra*.

FLIES AS LABORATORY ANIMALS

In this connection it should also be pointed out that, although flies are valuable laboratory animals in certain types of nutritional studies, their true potentialities in this regard have not been appreciated. The great strides made possible in genetics by the fast-breeding *Drosophila* can and probably will be duplicated in studies of nutrition and physiological function using flies as test animals. Even epidemiological principles are capable of demonstration or development or both in captive fly populations. The economic advantage over vertebrate laboratory animals is obvious and, with imagination, the utilization of flies for basic research is great. The incomparable adaptability of the house fly, for example, is proof that this animal is possessed of complex survival systems. The recognition and understanding of these mechanisms may be of inestimable value.

THE SPECIES OF FLIES IMPORTANT TO PUBLIC HEALTH

Species prevalence.—Many studies of flies in urban areas have been made in recent years. The results of several of these studies in the United States indicate that, out of 18 or more species, usually less than a third are important. The house fly, *M. domestica*, the green blow fly, *Phaenicia sericata*, and the black blow fly, *Phormia regina*, are without doubt the most prevalent. In well-sanitized northern communities favored by a short fly season, a very few species, such as these three, may represent the entire fly problem.

In the age of the horse and buggy, Howard reported that the house fly comprised about 98 per cent of the flies found inside homes (64). That this still obtains today is far more surprising, but Haines has reported (65), 50 years later, that the same species made up over 99 per cent of the flies collected in occupied houses in two small cities in southwest Georgia. He found also that this species was the only one occurring in all 20 classes of breeding media sampled during a 12-months study. Twelve additional species or species groups were found more restricted in their choice of breeding media and were very minor in importance inside homes.

The worldwide fly species distribution studies are too numerous to review in our limited space and are usually of only inferential importance to the

assigned topic of this review. Studies on this subject that are of particular interest are cited by bibliographic references (66 to 76).

Limited space also forces similar handling of the following topics of only ancillary interest to this review: species surveys for fly adults (2, 77 to 82); species surveys for fly larvae (2, 65, 83, 84, 85); identification of flies (2, 5, 67, 83, 86 to 110); dispersal and flight range (111 to 130); and studies on fly biology and control (84, 101, 112, 131 to 159).

POPULATION EVALUATION

Epidemiological evaluation of vector-borne disease depends on reasonably good statistics for such information as the disease incidence, levels in both host and vector of susceptibility, and of disease carrier state, and the population levels of the host and vector. No one doubts the importance of knowing the population level of the vector in disease transmission, but ordinarily the figures on which this is based are poor in spite of the great need. The difficulty of devising methods for the analysis of the many complex variables involved in the population dynamics of the vector has made methods very few and inadequate.

Since 1945 the grill (24) has been widely used for estimating fly populations, principally those of the house fly. The principle of grill sampling is that of estimating by counts on a maximum concentration grouping of the flies in their free association with naturally occurring attractants. Flies in this state are highly sensitive to changes and to extreme levels of several factors including those of their own population, the temperature, wind, sunlight, and the time of day. By restricting the sampling to optimum conditions, "index" data are obtained, which appear sound empirically and reflect base fly populations responsible for the magnitude of the grouping phenomenon.

Fairly close correlation has been shown between fly grill indices and incidence of shigellosis as has been mentioned elsewhere in this paper. Experience in grill technique has led some workers to consider the retention of the grill device only as a standard in training for "visual surveys," in which the trained inspector is able to make fly grill readings by rapid visual estimation in the absence of the grill itself [Welch & Schoof (160)].

West (2), Watt & Lindsay (8), and others have commented upon the unsatisfactory features of trapping flies to determine an index to density. The authors have often seen fly traps in which *Phaenicia* was dominant and *Musca* uncommon among the trapped catch, yet the reverse was the visual observation in the area where the trap was located. Schoof & Savage (161) noted this in their five-community study in which traps showed relatively lower *domestica* prevalences than did direct observation or grill counts.

One may well say that if no flies are present the trap will catch none and be perfectly correct. But what of the trap that catches none, or at most only a few flies, in the absence of fly control and in the observed presence of many

flies? The latter situation is by no means unheard of when trap catches are used as indices to fly density. The more usual situation is one in which catches are too small to show significant differences between treated and untreated areas. This often occurs when it is obvious to any observer that flies are relatively scarce in the treated as compared to the untreated area. In addition to the recognized facts that traps have to compete with naturally-occurring and usually more abundant attractants, and that the kind of bait used may largely influence the kind of flies caught, there is another fact which is often overlooked. It is simply that the catch above any particular bait can never be exactly comparable to that of traps in other areas using identical baits because of the influence of nonidentical adjacent attractants; the same is true for traps set in the same area at successive times and for the same reason. A relatively constant fly population will redistribute itself with mercurial dispatch as the result of any change in the condition of attractants, and in many instances the competing trap is never seriously in the contest. At most the catch of any trap is a measurement of the relative effectiveness of its bait's attractiveness and is without much value as an index because the populations on other attractants cannot be established. Actually the only time a trap is infallible is when it catches huge quantities of flies and reflects a high fly density; ironically enough such conditions are perfectly apparent without trapping.

THE PUBLIC HEALTH SIGNIFICANCE OF FLIES

THE PUBLIC RESPONSIBILITY

Acceptance of fly control and prevention as a public responsibility has been extremely slow even in the more prosperous sections of the United States. By contrast, mosquito control and prevention has a history of nearly a half century of public effort and is now so accepted that in California, State and local agencies expend about three and a half million dollars per year in mosquito abatement (162). According to Mulrennan & Sowder (163), "At present, Florida is spending a total of \$3,322,105 annually, under the supervision of the State Board of Health, to control mosquitoes and other arthropods of public health importance." All of the species mentioned as "arthropods of public health importance" are mosquitoes or other biting flies. That most of these funds are primarily for pest mosquito control is undeniable, yet in contrast, little money has as yet been authorized for fly control on either a vector or pest basis though flies are a major problem in most areas.

The control or prevention of flies requires such intimate intervention into the residential lives and habits of the people and the design of their agriculture and industries that satisfactory reductive methods have not been developed. The advent of DDT made possible a method of control which could overlook the basic need for drastic improvement in the handling of solid

organic wastes, but with the rapid development of house fly populations resistant to DDT and related compounds, the need for basic development of fly prevention and preclusion is again before us.

CONTROL OF FLIES AS VECTORS OF DISEASE AGENTS

When flies are important vectors of shigellosis bacteria, fly control needs no further justification than the disease. Any plan for control, short of emergency, should be well integrated into a diversified program of health services and environmental sanitation, since flies are not alone responsible for the transmission of the disease agent, and their eventual prevention rests secondarily upon correction of many other problems in environmental control.

The social significance of diarrheal disease has always been slight, because those attacked are in the lowest stratum of society, and the morbidity and mortality are chiefly infant. Were the disease one that struck indiscriminantly through our population as did the mosquito-borne diseases, more public support for fly abatement would probably be available. Under the possible conditions that may be postulated during disaster, flies may prove capable of carrying more disease agents than that causing dysentery. When every factor tends to favor such transmission, flies may demonstrate their proven ability to transmit the agents of some 30 different diseases, heretofore shown only under laboratory conditions (2, 5, 87).

CONTROL OF FLIES AS AGENTS IN MYIASIS

Although no justification of fly control for myiasis prevention in man has been made, large programs for myiasis prevention in animals have been well known in most countries producing livestock (5, 49, 83).

CONTROL OF FLIES AS PESTS

The question of the responsibility of public health agencies in the abatement of nuisances or pests is one of the more difficult facing us today. However, in an age when those who enjoy a high standard of living are spending progressively more and more for creature comforts, the eventual fate of public nuisances or pests cannot be other than in public responsibility. In California 48 local health departments received a total of 37,700 requests for assistance on vector problems during 1953 (164). Of these requests, over 36 per cent involved flies, as compared to 11,930 involving rats, the next most frequent category.

Because acceptance of the fly control problem, with its manifest difficulties, offers little tangible hope of success to the agency involved, few have undertaken this responsibility. On the other hand, even the Federal Government has emphasized the place of pest mosquito control in local health activities (165) and several States have long had major programs.

Foremost among the State health departments in the acceptance of fly

control as a public health concern is California, whose Bureau of Vector Control includes fly control as one of its major program elements. Its official statement (166) on "Discomfort (Nuisance) Control" is that:

While the underlying objective of vector control has always been that of emphasis upon control of proven vectors of disease, it has in practice been impracticable and we have come to believe it to be undesirable to confine the program strictly to disease control. Administratively and operationally, difficulties are encountered in attempts to obtain species sanitation, particularly when the vector is relatively innocuous as a pest, but coexists in areas with more obvious species which cause the public great discomfort. More and more the influence of economics and discomfort, expressed as nuisance, has become the chief basis for public support of vector control operations in this general field. The ever increasing demands of the public for high levels of comfort as well as sanitation, have invited the broadening of vector control to include the realm of environmental nuisance control. Both these activities have a solid legal basis as public health programs.

Following the lead of the California State Department of Public Health, the local health department of Santa Clara County, California, has established a Division of Vector Control which is currently engaged in an active program of fly control and prevention on a basis of investigation, consultation, training, and public education (85).

NEW PROBLEMS AND THE NEED FOR NEW CONTROL METHODS

Our prosperous and ever increasing population has created, and is rapidly aggravating, enormous problems in the handling of solid organic wastes, for which there seems to be no immediate prospect of solution. There are grave difficulties in store even for the water-carriage systems of large municipal areas, but solution of the problems of how to collect, store, and dispose of solid wastes are equally important to progress. In discussing the disposal problem, McGaughey of the University of California, stated in 1952 that "In Los Angeles County, for example, it has been estimated that within the next thirty or forty years all available land fill sites within a hundred miles of the city could be filled" (167).

Proposals for rapid aerobic composting are now being offered as a solution, with conservation of our organic matter by returning it to the soil as a wise foresight (167 to 170).

Stead (171) gave emphasis to six public health aspects of refuse disposal, with flies heading the list, followed by rodents, air pollution, water pollution, transportation nuisances, and lastly changes of topography through land use for disposal.

The direct relationship of fly population level to the status of solid organic waste disposal in communities was well demonstrated by Maier & Baker in their studies of sanitation in Rio Grande Valley towns in Hidalgo County, Texas (172). One of their graphs shows peaks of high fly population reflecting the improper disposal of vegetable canning and packing plant wastes in

one town while, by contrast, another town employing a sanitary fill has only a moderate and relatively constant fly population. By their presence and numbers, flies indicate the gravity of the disposal problem.

Almost every operation in the handling of garbage, fruit and vegetable cannery waste, meat packing waste, or manure makes fly production possible. Poor handling is obviously a large factor, but it should be emphasized that many industries, especially agriculture, have developed new fly problems along with newer designs for improved quality of products and increased production. As illustrations of the increase of fly problems with new designs, one may cite the use of individual cages for laying hens as compared to the open yard and litter system; the use of high value feeds for stockyard-penned steers and dairy herds as compared to old style feeds; disking cannery waste into agricultural land as compared to land fill methods; and home composting as compared to off-the-premises disposal. Problems have come even with new community design, by which suburban developments with schools and commercial centers are crowded between dairies, poultry ranches, riding stables, canneries and packing sheds. Economics and modern architecture combine to make screening a forgotten item in new schools of the growing suburbs, while the home owner strives to live more and more out-of-doors. Flies inevitably enter the picture as major pests, emphasizing the problems of waste disposal yet to be met by the community and its citizens (65).

The choices available to the suburbanite are these: (a) Combat the flies with every available insecticide in the home and be resigned to a minimum of success. The speed with which major fly sources will replenish the fly population is very discouraging. (b) Organize the community to force the neighboring agricultural and food industries to keep their fly populations within their confines or face court action. By zoning ordinances, attempt to curtail future expansion of the problem. Widespread rearrangments in the local economy can be expected, if these measures are truly successful. (c) Seek technical guidance toward intelligent industry and community action in solving the problems of solid organic waste disposal. Progress can be made toward a reasonable coexistence of agriculture and suburban living with a considerable gain in both public health and agriculture. The average community can go far toward the prevention of fly and rodent problems.

Space will not permit a review of the numerous papers dealing with methods of fly preclusion and with insecticides and their applications in the control of nonbiting flies, but those papers of particular interest are indicated in the bibliography (122, 173 to 184).

FLIES AS INDICATOR ORGANISMS

It is justifiable to regard flies in a community as indicator organisms, symptomatic of disposal problems and reflecting the sanitary level of the community, much as for years we have used coliform organisms as indicators of water potability. With this concept, control of flies with insecticides alone

falls into its proper place as a temporary palliative without lasting benefit.

The use of flies as indicators is, in fact, already in use. In the tomato canning industry, pomace or vinegar flies, *Drosophila* spp., are now used by sanitarians as indicators of the quality of tomatoes used by the canner (185). If broken or partly decomposed fruit are used, *Drosophila* will be present as insect parts in the canned product, leading to its condemnation because of the adulterant. As the problem also extends beyond the use of tomatoes of poor quality, *Drosophila* has to be controlled for its own sake (186, 187).

Combating the situation is first of all a matter of meeting squarely and honestly the disposal problem itself, seeking practical solutions rather than concentrating only upon the secondary fly problem. The task basically is one of remedying the design and management of organic waste handling, a goal more important than fly control for many reasons, among which conservation of nitrogenous material would seem to be foremost.

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VETERINARY AND MEDICAL ACAROLOGY¹

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INTRODUCTION

This review concerns ticks and mites of known veterinary or medical importance. Acarina that are normally parasitic upon vertebrates include those of greatest importance to the present subject. Parasitism of vertebrates has arisen among quite unrelated forms of Acarina; hence, with the exception of ticks, the families discussed here form a confusing assemblage rather than a natural group. Furthermore, since the tissues of many parasitic Acarina satisfy basic requirements for a wide variety of pathogenic microorganisms, including certain viruses, rickettsiae, bacteria, spirochetes, protozoa, and helminths, one must deal with a similarly wide variety of problems in microbiology. Finally, various degrees of complexity may characterize the ecologic relationships among an acarine species, its vertebrate host species, and a species of microorganism shared by both. For these reasons the problems of veterinary and medical acarology, with their breadth of scope, present a stimulating challenge to workers in many fields of basic and applied biology.

As an introduction to the great variety of ecologic problems encountered, certain facts are worthy of mention. During at least one stage in their life cycle, all ticks and certain mites are parasitic on or in terrestrial vertebrates. Since some of these parasitic forms attack human beings or domestic animals, they may act as direct causative agents of disease, or they may transmit pathogenic microorganisms. Certain parasitic species, not known to attack man or domestic animals, may play roles in the biological survival mechanisms of important pathogens by transmitting them among suitable wild hosts and by acting as reservoirs of infection. Some mites which are normally free-living may occasionally cause accidental infestations of man, while other nonparasitic forms may harbor the immature stages of parasitic worms. Examples of these and of more complex kinds of relationship will be considered in this review.

The review itself is selective rather than comprehensive. It aims neither at complete coverage of the literature, nor at a balanced presentation. It is intended to make the reader aware of the breadth of the subject and to present the reviewer's evaluation of certain aspects. The critical reader, appreciating the scope and diversity of veterinary and medical acarology, will understand this approach, while the bibliographer will find the review of little use.

¹ The survey of the literature pertaining to this review covers approximately the five years prior to January 1, 1955.

GENERAL CONSIDERATIONS

The Acarina form an order of the class Arachnida, and they possess the typical features of this class. For instructive and authoritative presentations of the classification and biology of Arachnida, consult Petrunkevitch (1, 2); his are the only phylogenetic studies of this class which appear sufficiently basic to merit serious consideration by zoologists. Within the order Acarina, the major categories (suborders and superfamilies) have yet to be grouped and defined on a wholly rational and defensible phylogenetic basis. For the best available modern account of Acarina, the reader should consult *An Introduction to Acarology* by Baker & Wharton (3). Among other desirable features, this book contains keys which are useful in assigning Acarina to families. Some students of the group might consider it unfortunate that Baker & Wharton (3) followed, in many respects, the classification of Vitzthum (4), but they were cognizant of the impossibility of producing a satisfactory phylogenetic classification at the time their book was being prepared. This fact in no way detracts from the value and usefulness of the book as a fundamental reference, and it is merely a reflection of the larval state of the science of acarology.

One who is interested in problems of transmission of disease by Acarina should acquaint himself with modern points of view in microbiology and epidemiology. The chapters in the books edited by Rivers (5) and Dubos (6) contain authoritative information on viruses, rickettsiae, bacteria, and spirochetes pathogenic for man. They also contain clear expositions of the quantitative concepts in microbiology which are essential to an understanding of current trends in this field and hence also in modern veterinary and medical acarology. In the case of protozoa and helminths, the reviewer has not encountered recent comprehensive books which meet the standards set by Rivers and Dubos. For a clear exposition of the modern view of epidemiology as a problem in medical ecology, the reader should consult Gordon (7). Current principles and practices in the control of communicable diseases of man, some of which are caused or transmitted by Acarina, are given in a recent publication by the American Public Health Association (8).

The problems of veterinary and medical acarology to be reviewed here are conveniently discussed as they pertain to members of two families of ticks and several families of mites; they are presented accordingly.

TICKS

FAMILY ARGASIDAE (ARGANTIDAE): SOFT-BODIED TICKS

Three genera of this family contain species known to be of medical or veterinary importance. Certain species of *Ornithodoros* are natural hosts and vectors of the spirochetes which cause relapsing fever, and a few ticks of this genus produce venomous "bites." *Argas persicus* is not only a frequent parasite of domestic fowl, but is also a vector of fowl spirochetosis in certain

localities. The third genus of importance, *Otobius*, includes *O. megnini*. The immature stages of this species, commonly known as the spinose ear tick, are important parasites of cattle, but the adults do not feed at all.

As a result of their important role as biological vectors of relapsing fever, *Ornithodoros* ticks probably deserve most emphasis. Before these ticks are discussed as vectors, however, several points in their biology will be mentioned. The well-known ability of *Ornithodoros* to survive under conditions of starvation and low ambient humidity indicates that these ticks possess remarkable adaptations for conserving water. Part of this adaptation depends upon the presence in the epicuticle of a thin layer of wax which greatly reduces water loss through the integument. Browning (9) observed that the spiracles possess an efficient mechanism for closing. In further studies Browning (10) found evidence of a third mechanism, and one which depends upon the activity of the epidermal cells. Unfed nymphs of *O. moubata* are apparently able to extract water from moist air (relative humidity 95 per cent) and to restrict the rate of water loss in dry air. The ability to regulate water balance is profoundly affected by carbon dioxide tension. It is known that a concentration of 5 per cent causes opening of the spiracles. Browning (10) found that concentrations of carbon dioxide in the range of 30 to 45 volumes per cent, sufficient to cause anesthesia, affected the ability of the epidermal cells to abstract water from the air and to restrict its loss in dry air. Further studies are necessary to clarify the mechanisms underlying these effects of carbon dioxide upon these functions of the epidermal cells.

Observations on several aspects of the biology and bionomics of certain species of *Ornithodoros* have been reported during recent years: *O. tartakovskyi* by Baltazard *et al.* (11), *O. graingeri* by Heisch & Harvey (12), *O. hermsi* by Longanecker (13), and *O. brasiliensis* by Davis (14). From the latter species Davis (14) recovered a spirochete which he named *Borrelia brasiliensis* and which produces a relapsing infection in white mice.

Ornithodoros and relapsing fever.—Many recent textbooks unfortunately contain confusing, noncritical, and erroneous accounts and tabulations of these ticks and of the spirochetes transmitted by each species. The only correct recent account known to the reviewer is that by Davis (15) in the book authored by Mackie, Hunter & Worth (16). The complexity of the problem of these ticks as vectors of relapsing fever is illustrated by the following facts: (a) the various "species" of *Borrelia* cannot be separated on the basis of morphology or tinctorial properties; (b) they have not been found to multiply in acellular media; however, they multiply within developing chick embryos; and (c) adequate criteria for species separation, based on the biological characteristics of *Borrelia* spp., have not been firmly established.

With respect to the problem of species differentiation on the basis of biological properties, much critical pioneer work has been accomplished by G. E. Davis in the United States. The underlying principle is the demonstration of tick-spirochete specificity in the case of certain combinations of

species of *Ornithodoros* and *Borrelia*. The classical observations of Davis (17) showed the existence of specific relationships between *O. turicata*, *O. hermsi*, and *O. parkeri*, and their respective spirochetes. For example, *O. turicata* was able to transmit only its own spirochete and was unable to transmit spirochetes derived from the other two species of tick; similar specificity was observed for *O. hermsi* and *O. parkeri*. Recent studies by Davis (18) concerned two American species, *O. turicata* and *O. talaje*, which occupy the same habitats in nature and presumably often feed on the same individual hosts. Strains of spirochetes from each species of tick have been established in appropriate laboratory animals. When Davis (18) fed spirochetes from *O. turicata* to ticks of both species, only *O. turicata* became infected as a biological vector. Spirochetes from *O. talaje*, under analogous conditions, failed to infect *O. turicata*, but became established in *O. talaje* and were transmissible by it. Certain other combinations of ticks and spirochetes have shown similar degrees of specificity when tested under appropriately controlled conditions. The principle of a specific tick-spirochete relationship has been used by Davis (19), in combination with other experimental biological criteria, as an aid in identifying the two closely related and morphologically similar tick species, *O. turicata* and *O. parkeri*.

A specific relationship between a species of arthropod and a species of microorganism, in whose biological survival mechanism the arthropod plays an essential role, is doubtless of fundamental evolutionary significance. Tick-spirochete specificity, studied intensively and extensively by Davis, is of such broad implication that it deserves to be more widely recognized and appreciated.

The interepidemic reservoirs of the relapsing fevers pose a question of epidemiologic importance which involves consideration of the fundamental biological survival mechanism of *Borrelia* species and of the roles played by arthropods as hosts to the spirochetes. It is generally accepted that *Ornithodoros* ticks function as reservoirs as well as vectors of the respective *Borrelia* species which they transmit. The evidence includes numerous clear-cut demonstrations of longevity of ticks and of transovarial passage through several generations in a number of species of ticks. With respect to louse-borne relapsing fever, however, it is obvious that lice are not the reservoir, and that lack of critical data makes it impossible to do more than speculate on the possible role of human beings as reservoirs of the spirochetes. It is attractive to hypothesize that tick-borne relapsing fever, occurring in a louse-infested human population, might become louse-borne and continue as such until the epidemic, for unknown reasons, ceased. The observations of Baltazard *et al.* (20) bear directly upon this problem. They reported definite experimental evidence, obtained from human volunteers infected with strains of spirochetes from *O. erraticus*, indicating that human beings did infect body lice and that the lice transmitted the infection to susceptible volunteers. During repeated serial transmission from man to man by means of lice, there was no apparent modification in the properties of the spiro-

chetes. These observations of Baltazard *et al.* (20) constitute a valuable contribution to the problem, and it is desirable that further studies be made of the possible relationships between louse-borne and tick-borne relapsing fevers.

Further recent advances in our knowledge of *Ornithodoros* will be reviewed as they pertain to particular species.

Ornithodoros moubata.—This important vector of relapsing fever in Africa has been studied for many years. Its recorded distribution has been mapped and discussed by Leeson (21). The distribution appears to follow lines of communication. This is perhaps attributable to the fact that Africans often purposely carry ticks with them when they are likely to be away from home for long periods. They are believed to allow the ticks to bite them so as not to lose their immunity to relapsing fever. Although the immunologic effectiveness of this practice has not been evaluated, the possible epidemiologic implications are obvious.

For recent information on *O. moubata* as a vector in East Africa, consult Heisch (22). A detailed study of this species as an experimental vector of *Borrelia duttoni* was made by Burgdorfer (23) with particular reference to such problems as the distribution of the organisms within each stage of the tick and their egress via salivary secretions, coxal fluid, or both.

Weyer (24) showed that when the rickettsiae of epidemic or of murine typhus are injected into the hemocoel of *O. moubata* viable organisms can be demonstrated for considerable periods thereafter. This sort of observation is of interest mainly to the experimentalist. For example, the ability of certain species of *Ornithodoros* to maintain a number of pathogens for appreciable periods of time makes them useful for the purpose of transporting various strains of viruses or rickettsiae. One would naturally avoid the use of ticks whose microbiologic history was unknown. It is of further interest that Schlossberger & Langbein (25) demonstrated the survival, probable multiplication, and transovarial passage of *Leptospira icterohaemorrhagiae* in experimentally infected *O. moubata*. These examples (*Borrelia* sp., *Rickettsia* spp., and *Leptospira* sp.) illustrate the versatility of this tick's tissues as a suitable environment for microorganisms whose requirements are quite diverse.

The observation that *O. moubata* is essentially an inhabitant of African dwelling places poses the question of where the tick lived before it became associated with man. Walton (26) recorded personal observations in Tanganyika of wart-hog and porcupine burrows which were heavily infested with this species. He has summarized an increasing amount of evidence that *O. moubata* is associated with large burrowing animals over a wide area of eastern Africa. Further studies of this sort are needed in the case of several other "house-haunting" arthropods.

Natural populations of *O. moubata* are of interest with respect to the ecology of relapsing fever. Phipps (27) studied the degree of infestation of dwellings in various places in Tanganyika. In comparing the infestations,

he used the mean log (catch +1) for purposes of statistical analysis, and noted a correlation between this value and the percentage of positively infested houses in different parts of the country. Prof. C. B. Williams of Rothamsted introduced the use of mean log (catch +1) as a tool for the analysis of enumeration problems in ecology. It is a technique for estimating a geometric mean, and Williams found it to be useful in the analysis of data obtained from insect traps. In the reviewer's opinion it merits further application to the complicated problems of comparing ectoparasite infestations. With respect to the control of *O. moubata*, numerous workers have demonstrated the effectiveness of the gamma isomer of hexachlorocyclohexane, if used in conjunction with appropriate environmental measures. Examples of such work are the reports by Knowles & Terry (28), Annecke & Quin (29), Teesdale (30), and Holmes (31).

Ornithodoros erraticus—This species presents an important and difficult problem in speciation. It occurs in nature in two so-called forms, large and small, as noted by Baltazard *et al.* (32). One or both of these forms are found in Spain, North Africa, Iran, and in or near the Rift Valley of Kenya and Uganda. According to Davis (15, p. 101), *O. erraticus* was originally described from a specimen of the small form, and the name *O. maroccanus* is available for the large form if the two are ultimately recognized as distinct species. According to Baltazard *et al.* (32), the large form transmits *Borrelia hispanica*, while the small form transmits *B. crocidurae*, *B. microti*, and *B. merionesi*; these relationships were accepted by Davis (15). Blanc (33) studied the problem in Morocco, where the large form was observed in the northern portion and the small form in the southern part of the country. These investigators noted, however, a zone of contact in which both forms were found together in the burrows of gerbils. Chabaud (34) recently reviewed the problem of multiple races in *O. erraticus* and presented observations of his own. On the basis of hybridization experiments, he concluded that the two forms could not be elevated to the status of two distinct species. If one considers problems in other fields of biology, however, it is clear that the results of reciprocal hybridization experiments in the laboratory are not necessarily conclusive, in themselves, with respect to speciation, for such experiments may easily fail to include mechanisms for reproductive isolation of populations which live together in nature. Thus it seems that additional studies of other biologic aspects of the *O. erraticus* problem are necessary for a critical evaluation of the species status of the small and large forms.

FAMILY IXODIDAE: HARD-BODIED TICKS

This family comprises several genera containing species which may be natural hosts to one or more species of microorganisms, including viruses, rickettsiae, bacteria, and protozoa. Recent years have seen outstanding contributions to our knowledge of the taxonomy, biology, and ecology of hard ticks, but these would not benefit from any attempt at an abbreviated presentation. The important but imperfectly understood problem of tick

paralysis cannot be considered adequately in the present review. The agent of tularemia has been recovered from an ever increasing number of species of ticks, and its isolation from such sources is no longer considered noteworthy. A similar remark is perhaps applicable to the rickettsiae of Q fever; although ticks possibly function as reservoirs of the agent, and although they are good candidates for potential vectors, yet their actual importance in transmitting the agent to animals and to man remains to be demonstrated conclusively.

Four topics have been selected for review herewith: ticks and Rocky Mountain spotted fever, tick-borne hemorrhagic fevers, tick-borne encephalitides, and resistance in ticks to insecticides.

Rocky Mountain spotted fever.—Recent outstanding studies by Price (35 to 40) have enhanced considerably our understanding of ticks as hosts and vectors of *Rickettsia rickettsii*. Two broad problems which have received attention are (a) the effect of passage in ticks on certain properties of the rickettsiae, particularly virulence, and (b) the role of ticks and of their mammalian hosts in the biological survival mechanism of the rickettsiae. Orientation toward modern principles and quantitative concepts of microbiology is essential to an understanding of this type of problem, and the results of Price's studies exemplify a fruitful application of such an approach.

As a basis for the understanding of Price's work with ticks, it is necessary to present certain of his findings which concern the rickettsiae. By means of comparative studies in guinea pigs and embryonated eggs, the numbers of rickettsiae required to infect and kill eggs and to infect guinea pigs were determined. In other words, the sensitivity of these experimental hosts was compared quantitatively, and the reproducibility of their responses was determined. Thus the available experimental tools for detecting and counting viable *R. rickettsii* were subjected to critical evaluation.

Virulence, a concept which is often referred to in vague terms, was rigidly defined (36, p. 248) and was studied by observing the responses of guinea pigs. The following points were noted: presence, amount and duration of fever, scrotal reaction, per cent fatality, and persistence of demonstrably viable rickettsiae in the animals. As is the case with many biologic properties, virulence was found not to be an all-or-none phenomenon, but to follow a biological gradient. Price was able to classify his strains with respect to virulence into four groups, designated R, S, T, and U. Types R, S, and T multiplied and produced fever, while U neither multiplied in guinea pigs nor produced fever. Types R and S produced scrotal reaction, but T did not. At the dosage tested, R strains were the only type which killed guinea pigs. All four types multiplied in the yolk sac of embryonated eggs. It should be emphasized at the outset that U did not multiply in guinea pigs and that serial propagation in eggs did not make U strains able to propagate in guinea pigs.

A further basic point is the fact that R, S, and T strains were found to exist in an avirulent phase. If such avirulent phase organisms were injected into a guinea pig, they did not multiply, and the animal merely developed

antibodies, the response thus being analogous to that to a vaccination. On the other hand, if avirulent phase rickettsiae were injected into embryonated eggs, they not only multiplied, but they also acquired a degree of virulence which corresponded to that of the parent strain, as judged by the responses of guinea pigs to inoculation with standard doses. Hence, in order to characterize an unknown strain, and to distinguish a U type from an avirulent phase of the R, S, or T types, the agent must be studied in embryonated eggs and also in guinea pigs before as well as after yolk sac propagation. With these aspects of Price's work in mind, it is appropriate to review his findings concerning ticks and spotted fever rickettsiae.

(a) Reactivation of virulence in ticks: Previous workers had noted that strains of *R. rickettsii* recovered from ticks differed widely in their ability to cause overt disease in guinea pigs. It had been known for more than 25 years that if individuals of *Dermacentor andersoni*, infected with a fully virulent strain, were refrigerated for several months, a suspension of the triturated ticks produced immunity, but no disease or only insignificant disease, in guinea pigs. If previously unfed infected ticks were given a blood meal and then tested in guinea pigs, the animals suffered an overt and often fatal infection. Although this phenomenon was believed to represent "reactivation" of virulence, this interpretation was open to question, since the dosage of rickettsiae was unknown, and hence the possibility that "reactivation" was attributable merely to multiplication of rickettsiae in the ticks had not been ruled out. Price's quantitative experiments showed that the rickettsiae could be made virulent by leaving the infected ticks at 37° C. for 48 hr. or by passing them once through developing chick embryos. The observations were reproducible, and the dosage of rickettsiae was determined by appropriate titrations. The avirulent phase, having been demonstrated to occur in adult *D. andersoni*, was also found in the eggs, larvae, and nymphs of laboratory-reared ticks from an infected stock.

For a concise presentation of the properties of the virulent and avirulent phases of *R. rickettsii*, the reader is referred to Price (35, Table 2). The phenomenon of reactivation of virulence provides a clearcut demonstration of an important effect of an arthropod host upon an infectious agent within its tissues. Not only can multiplication or developmental change occur within a biological vector, but such important properties as virulence for a vertebrate host may also be demonstrably affected. Although the bearing of reactivation upon the detectability of rickettsiae must be taken into account in conducting a survey for spotted fever in ticks in nature, the broad biologic implications of this phenomenon are of greatest interest.

(b) Effects on *Rickettsia rickettsii* of passages in ticks, and in rabbits: For many years it was known that strains of *R. rickettsii* recovered from rabbit ticks, *Haemaphysalis leporis-palustris*, collected in the western United States, produced only mild disease in guinea pigs. Price's titrations in embryonated eggs showed that this is not merely a question of dosage, but that these strains multiply poorly in guinea pigs as judged by the relatively

low titers achieved in various tissues. He also found that (i) passage in *D. andersoni* of strains recovered from *H. leporis-palustris* did not enhance their virulence, and (ii) passage in *H. leporis-palustris* of fully virulent strains did not decrease their virulence.

In the mammalian host, however, it appeared that the virulence of strains could be modified temporarily. When a type R strain was carried through continued spleen passage in cottontail rabbits, a strain of the S type was obtained after 8 to 13 passages. In another series of experiments initiated with a fully virulent strain, organisms of the T type eventually were obtained. These changes were temporary, however, for when these rabbit-passed strains were subsequently passed serially in guinea pigs, their properties reverted to those of the original, fully virulent, R type. These results in general suggest that the virulence of *R. rickettsii* can be modified, at least temporarily, by passage in cottontail rabbits, but not by changing the species of tick host in which multiplication occurs.

(c) Interference between rickettsial strains: The phenomenon of interference is well known in the case of many viruses, and Price (37, 39) demonstrated it for rickettsial infections in mammalian hosts. For its implications with respect to ticks, the reader should note experiments summarized by Price (37, Table III). In a typical experiment with *D. andersoni* nymphs, 100 individuals infected with low-virulent T type rickettsiae and two individuals infected with highly virulent R type organisms were fed simultaneously on a Columbian ground squirrel. Three days later 100 noninfected laboratory-reared nymphs were placed on this squirrel and allowed to feed to repletion and molt to adults. When these previously noninfected ticks were tested subsequently, they were found to contain only the low-virulent T type strain. In analogous experiments, in which ticks harboring a highly virulent strain predominated, the noninfected ticks became infected only with R type rickettsiae. Other experiments, described in the same paper (37), demonstrated that interference occurred in the mammals; nothing is known concerning its possible occurrence in ticks.

Although Price realized that interference required further study, he clearly indicated its potential implications with respect to the epidemiology of spotted fever. For example, Parker noted 30 years ago that, in certain limited localities, strains having high or low virulence persisted for a number of years, and it is quite plausible to suppose that interference may be a factor involved in maintaining such situations.

(d) Role of ticks in the biological survival mechanisms of *Rickettsia rickettsii*: The ecology of Rocky Mountain spotted fever in the western United States differs in several important respects, especially tick species and mammalian hosts, from that in the eastern part of the country. The early studies of Ricketts, Wolbach, Parker, and the latter's associates, established the principal features of the ecologic complex in the Rocky Mountain region, where the infection chain involves trans-stadial and transovarial passage of the organism in *D. andersoni*, and the winter season is spent in

the nymphal or adult stages of this tick. New lines of infection can be started when infected and noninfected ticks feed simultaneously on a suitable host. The rabbit tick, *Haemaphysalis leporis-palustris*, which apparently never bites man, may transmit mild strains among rabbits, but its relative importance in maintaining strains of the type usually recovered from human beings has not been established.

The ecology of spotted fever in an eastern area has been studied in Maryland by Price (40). He conservatively limited his inferences to the localities studied and carefully made no claim that the observations might be more widely applicable. It has long been known that the main vector to man in the eastern states is *Dermacentor variabilis*, that the larvae and nymphs feed commonly on *Microtus pennsylvanicus* and sometimes on other small mammals, but never on dogs or man, and that dogs are frequent hosts for the adults. Relatively little was known about the roles of these mammals as hosts for spotted fever rickettsiae. Price (40) demonstrated the potential importance of *Microtus* and of dogs, in that they can serve to initiate new lines of infection in ticks when infected and noninfected *D. variabilis* feed simultaneously on the same animal. Low virulent strains of the T type were the only ones encountered during his study, and these appeared to die out in infected adult *D. variabilis* after 14 to 16 months.

Quantitative studies of transovarial transmission showed that 30 to 40 per cent of infected female *D. variabilis* transmitted the rickettsiae to at least some of their eggs, but the number of infected eggs differed widely among various females. In every instance studied, in which an infected egg produced a larva which ultimately gave rise to a viable adult tick, the adult was infected. In other words, trans-stadial transmission occurred in 100 per cent of infected ticks. Price postulated that the rickettsiae are maintained in nature by a dual survival mechanism, in which transovarial transmission plays a role which is supplemented by passage from infected to noninfected ticks when they feed simultaneously on a suitable host. (Previously published diagrams and descriptions which incorporated this mechanism were based on its plausibility rather than on concrete experimental observations.)

It has not been possible to review all of the important and interesting information contained in Price's papers, and these should be consulted in the original. The mechanisms underlying reactivation of virulence are now under investigation in Price's laboratory, and it is to be expected that further basic results will be forthcoming.

Tick-borne hemorrhagic fevers.—Medical investigators in the U.S.S.R. during the past 20 years have encountered a group of infectious diseases, characterized clinically by an acute hemorrhagic syndrome and epidemiologically by a distinct geographic localization of each of the separate diseases. Several of these hemorrhagic fevers are known or believed to be caused by distinct viruses, and four of these are believed to be transmitted to man by ixodid ticks. Hence a discussion of the problem is included in the present re-

view. The following remarks are based on a recent, critical, well documented review by Gajdusek (41).

Crimean hemorrhagic fever is a rural disease with seasonal incidence, occurring sporadically in agricultural workers from April through September. The specific virus has been recovered from *Hyalomma marginatum* derived from two sources: nymphs taken from the European hare, *Lepus europaeus transsylvanicus*, and starved adult ticks collected in the steppe region. In places where the disease occurs in man, the population of ticks reaches its peak in July, shortly before the peak in human cases occurs. Further information on the habits of the ticks and the behavior of the virus in their tissues and its mode of transmission is needed in order to clearly establish the role of ticks as vectors.

Omsk hemorrhagic fever, which occurs in the Baraba Steppe of the Omsk Oblast in Siberia, resembles the Crimean disease in its clinical and epidemiologic features. The tick vector is suspected to be *Dermacentor pictus*. The Russian workers state that this tick has transmitted the virus while feeding on experimental hosts. The recovery of the virus from wild caught ticks has been reported. The peak in seasonal activity of the ticks precedes the peak in human cases by an interval corresponding to the period of incubation in man, and *D. pictus* frequently bites man. A common wild rodent, *Microtus gregalis*, is a frequent host in nature of *D. pictus* and of the virus.

Uzbekistan hemorrhagic fever has been reported from many parts of Soviet Central Asia, but mainly from Uzbek S. S. R. The vector is believed to be *Hyalomma anatolicum*, a tick which frequently is found on cattle, horses, and sheep and within farm buildings. If often attacks man, and it has been taken off patients sick with the disease. Its maximum prevalence occurs during June and July. The virus of Uzbekistan hemorrhagic fever has been recovered from 17 of 22 suspensions made from pools of this tick. The present reviewer is unable to find a statement concerning experiments with transmission during feeding. The virus has been recovered also from two other species of tick, *Hyalomma detritum* and *Rhipicephalus turanicus*; although these species also attack man, their role as vectors has not been established.

Bukovinian hemorrhagic fever occurs in the wooded Carpathian foothills of Northern Bukovina, formerly a part of northern Roumania. Although a viral etiology is suspected, the etiologic agent has not been positively identified. Until this has been done, a specific vector relationship cannot be demonstrated. In the meantime, *Ixodes ricinus* is suspected as a possible vector for the following reasons: many patients report tick bites, and lesions consistent therewith are found on these patients; *I. ricinus* is frequently found on the patients, and it is common in the forest areas where the disease is presumably acquired.

These hemorrhagic fevers which are known or suspected to be transmitted by ticks are of particular interest because they represent a previously

unknown group of tick-transmitted diseases. They are mentioned here because papers in the English language on acarologic subjects have contained little information and Gajdusek's review (41) of the subject is a most valuable contribution.

Tick-borne encephalitides.—During recent years it has become increasingly apparent that a number of tick-borne neurotropic viruses of Europe, Russia, and northern Asia are very closely related, and perhaps indistinguishable (5, pp. 242–47). When Pond, Russ & Warren (42) studied a portion of the problem by the available techniques of modern virology, their results lead them to suggest that Russian spring-summer encephalitis and louping ill be considered as strains of a single virus. Further information in a series of articles in the *Bulletin of the World Health Organization* by various authors (43) concerns a so-called virus meningo-encephalitis in Austria and Slovenia. An additional important publication is a recent book in Russian, edited by Smorodintsev (44), concerning so-called diphasic virus meningo-encephalitis in European Russia.

Although the problem of these tick-borne neurotropic viruses has not yet been evaluated fully, certain generalizations may be of interest to acarologists: (a) In several parts of Europe and in portions of the western, central and eastern Union of Soviet Socialist Republics, several different clinical pictures are produced by viruses which are perhaps indistinguishable and possibly identical. (b) Certain of these clinical pictures are associated with characteristic and distinctive epidemiologic features. (c) *Ixodes ricinus* apparently transmits virus meningo-encephalitis of man in Slovenia (43), diphasic meningo-encephalitis in European Russia (44), and louping ill of sheep in various parts of Europe (5). (d) The tick vector of spring-summer encephalitis is suspected to be *Ixodes persulcatus*, and there is evidence that other species may also be involved.

Resistance in ticks to insecticides.—Recent years have been marked by reports of strains of ticks which are resistant to the newer insecticides. Examples include the resistance of *Boophilus decoloratus* in South Africa to the gamma isomer of hexachlorocyclohexane (lindane), studied by Fiedler (45) and by Whitnall *et al.* (46), and the resistance of *Boophilus microplus* in Australia to the same substance, reported by Hitchcock (47). The following notes are based on the account by Whitnall *et al.* (46). In the case of *B. decoloratus*, arsenic-resistant ticks were noted in a certain district of South Africa during 1938 to 1939. BHC was at first effective in killing these ticks, but later it was found to be ineffective on certain farms. The ticks were shown to be actually resistant to BHC, and they were still arsenic-resistant. No BHC-resistant *B. decoloratus* were found that were not also arsenic-resistant. Toxaphene and chlordane were ineffective against the BHC-resistant ticks, but they controlled BHC-sensitive ticks in the laboratory. It is to be expected that further examples of this general phenomenon will be encountered. Intensive study should be made of the basic as well as the applied aspects of this important problem as it relates to ticks and other Acarina.

MITES

The mites to be discussed in this review are grouped by Baker & Wharton (3) in four suborders of Acarina: (a) Mesostigmata, including blood-sucking and "parasitoid" mites; (b) Trombidiformes, including chigger mites; (c) Sarcoptiformes, including the mites causing scabies and mange; and (d) Oribatei, including the free-living mites which are intermediate hosts of certain tapeworms.

MESOSTIGMATIC MITES

Three general problems pertaining to these mites will be considered: (a) blood-sucking bird mites as possible vectors of neurotropic viruses, (b) the rat mite as a possible vector of pathogens, and (c) the mouse mite and rickettsialpox.

Blood-sucking mites of domestic and wild birds have been virtually accepted for some years, by a number of workers, as vectors to avian hosts of certain arthropod-borne neurotropic viruses in North America. Recent reports of critical field and laboratory studies in three localities by different groups of competent investigators (48, 49, 50), however, have furnished no convincing nor conclusive evidence of the importance or essentiality of bird mites as vectors of the viruses of western (WEE) or of eastern equine encephalomyelitis (EEE), or of St. Louis encephalitis (SLE). Inability to reproduce or confirm the experimental transmissions and transovarial passage of SLE virus, reported for *Dermanyssus gallinae* by Smith *et al.* (51, 52, 53), remains unexplained. In the case of *D. gallinae* and *Bdellonyssus sylviarum*, the observations of Reeves *et al.* (48) in California gave no conclusive evidence of long-term infection of these mites with SLE or WEE viruses, or of biological transmission of these agents. As a result of laboratory studies of *D. gallinae*, *B. sylviarum*, and *Bdellonyssus bursa* as possible vectors of EEE and WEE viruses, Chamberlain & Sikes (49) concluded that any role played by these mites in perpetuating these viruses in nature must be a minor one. Sulkin *et al.* (50), as a result of their field and laboratory studies, concluded that mites of wild birds or of chickens play no essential role in the epidemiology of WEE. The recovery of strains of these neurotropic viruses from mites taken off domestic or wild birds is now believed to indicate merely that the mites fed upon viremic birds shortly before they were sacrificed for detection of virus.

The tropical rat mite, *Ornithonyssus bacoti*, has not yet been demonstrated to be an important natural vector of human pathogens. It has, however, proved to be a useful laboratory tool in several areas of biological research. Gorirossi (54) made a valuable study of the mouth parts and associated musculature, and she postulated a feeding mechanism consistent with the probable functions of these structures. Cross (55) described a method whereby these mites are induced to ingest blood which is not confined under a membrane. This potentially useful technique should enable the performance of quantitative feeding experiments with pathogenic microorganisms,

and it might be useful in studying the nutritional requirements of these and related mites.

Studies of *O. bacoti* as an experimental vector of filariasis among cotton rats have been most fruitful. Outstanding among these are the studies recently summarized by Bertram (56). The three organisms, mite, filaria, and cotton rat, provide an excellent model for laboratory investigations in experimental epidemiology.

Hopla (57) reported various examples of biological transmission of *Bacterium tularense* by *O. bacoti* under experimental conditions. Philip & Hughes (58) found that *O. bacoti* could serve as an experimental vector of rickettsialpox. Schwab *et al.* (59), in studying this mite as a possible experimental vector of a strain of Coxsackie virus, obtained no conclusive evidence of an important role of this species in transmitting the agent which they studied.

The behavior of *O. bacoti* and of related blood-sucking rat mites as hosts to the rickettsiae of murine typhus is a moot question. In the reviewer's opinion, a critical evaluation of the problem would necessitate the application of modern techniques for detecting and identifying rickettsiae, in combination with modern methods for experimentation with infectious agents in arthropods.

Allodermanyssus sanguineus, a parasite of house mice, was demonstrated in 1946 as a vector of rickettsialpox to man. Nichols *et al.* (60) provided an interesting analysis of the relationship of the habits of the mouse and of the mite to the spread of rickettsialpox, the problem being presented as one in medical ecology. Observations on the life cycle of *A. sanguineus* were made by Fuller (61), but he was unable to establish a laboratory colony. Satisfactory colonization of this species would be a desirable accomplishment in order to provide suitable material for studies of the biology of the mite itself and of its role as biological vector under controlled conditions.

TROMBIDIFORM MITES

From the standpoint of disease transmission, chiggers (Family Trombiculidae) are probably the most important group of mites. Certain species are known to be vectors of scrub typhus; hence considerable attention has been given to these mites during recent years as a result of the importance of the disease during World War II. An authoritative account of scrub typhus is that of Smadel in *Viral and Rickettsial Infections of Man* (5). Much information on chiggers was presented in the comprehensive books published during 1952 by Womersley (62), Wharton & Fuller (63), and Fuller (64). Certain recent advances not cited by these authors are discussed in the present review according to three topics: (a) taxonomy of chiggers, (b) chiggers as vectors, and (c) control of chiggers.

Taxonomy of chiggers.—Since chiggers are most easily found when they are feeding as larvae on vertebrate hosts, most of the currently known species

have been described and figured in the larval stage only. Until these known forms have been correlated correctly with nymphal and adult stages (a formidable task), any definitive concepts of relationships among the species and genera of Trombiculidae will be difficult to defend. Recent studies of the fauna of certain regions or political areas include the following: a monograph of the species of the Pacific region by Womersley (62); a check-list for Asia and Australia by Gunther (65); systematic accounts of Japanese chiggers by Sasa & Miura (66) and by Sasa & Jameson (67); a preliminary account of chiggers of southern Korea by Jameson & Toshioka (68); studies of certain Malaysian forms by Audy and others (69); and descriptions of new species from Ruanda-Urundi by Jadin & Vercammen-Grandjean (70).

The existence of a rich chigger fauna on the African continent is indicated by the studies of Lawrence (71) and of Jadin & Vercammen-Grandjean (70). The wealth of the Oriental fauna has been clear for some years, and many species undoubtedly remain to be described. It is to be hoped, however, that important problems in speciation will be taken up by patient, competent, imaginative, experimentalists. For example, the taxonomist, whether he realizes it or not, is continually faced with the problem of evaluating the statistical and biological significance of variation. Womersley, during the 1940's, introduced and developed the practice of measuring certain structures of larvae and deriving statistics therefrom, these statistics being estimates of corresponding population parameters. This procedure has been applied widely in the study of larval chiggers, and Womersley has studied the statistical significance of differences in means computed from rather large series of specimens. Despite the results of a test of statistical significance, however, one may be quite uncertain as to the biological significance of observed differences, and it is the biological problem in which one is interested. Examples of difficulties of interpretation are provided by the observations of Richards (72, 73) concerning the common harvest mite, *Trombicula autumnalis*, in Great Britain, and by the problem of morphological definitions of the nominal species, *T. akamushi* and *T. deliensis*. In the case of these important species, it is not known how much variation may be expected among the sibling larvae produced by a single female. Lacking this elementary information, the student of living things finds difficulty in being certain what he is dealing with.

Such basic problems in speciation are amenable to a carefully combined field and laboratory approach. They should be attacked from the standpoint of modern experimental biology, but until this is done, our concepts of common and important species will lack a sound basis, and much of chigger taxonomy will suggest the application of philatelic thinking to populations of living organisms.

Further biometric studies of chiggers might well include the methods of statistical analysis developed by Mahalanobis and illustrated by Rao (74), the purpose being to classify different groups of individuals in the form of a significant pattern, and to describe quantitatively their similarities and

differences as groups, in an effort to gain some insight into their probable evolutionary history.

Chiggers as vectors.—The most important mite-borne disease of man is scrub typhus. Recent developments of particular interest include the demonstration of previously unknown or little studied foci of the infection in Korea, Japan, and nearby islands; the probable vector role of species other than the classical vectors; and the demonstrated effectiveness of newer chemicals against vector species.

If human beings are not essential to the maintenance and perpetuation of a particular infectious agent as a species, the absence of recognizable infection in man does not necessarily indicate the absence of the agent. Scrub typhus is a case in point. The existence of foci of infection in certain areas in Japan has been known for many years. During 1948, however, the presence of a previously unrecognized focus near Mount Fuji was confirmed by an outbreak of the disease among United States Army personnel; this was described by Thompson (75). The cases occurred during October and November, as opposed to the usual seasonal incidence during summer months in classical foci on Honshu. Four species of *Trombicula* were taken from wild rodents in this region, but *T. akamushi* was not found (76). The persistence of infected vectors in this focus was demonstrated during October, 1953, by the occurrence of cases among United States Marines; this latter episode was reviewed by Traub (77) and Fuller & Smadel (78).

Scrub typhus was recently identified on the Izu Shichito Islands, a group located off the southern coast of Honshu. The situation has been described by Yosano and others (79), and the nonclassical occurrence of human cases during the period November through January is emphasized by application of the name "Winter Scrub Typhus." The classical vector species were not found. Evidence incriminating *Trombicula scutellaris* was summarized by Sasa (80). This species was found naturally infected with the causative rickettsiae; it was shown to attach to man under experimental conditions; and its abundance in the larval stage was associated in time and place with that of human cases of the disease. Conclusive evidence of transmission by *T. scutellaris* during feeding has not yet been reported.

From these observations in Japan and nearby islands, it is clear that previously unrecognized foci of scrub typhus are sufficiently important to be reckoned with, that the disease may occur during autumn and winter months, and that species other than the classical vectors are strongly suspected as capable of transmitting the infection to man. Similar points emerge from recent observations of scrub typhus in Korea, as noted by Traub (77) and Fuller & Smadel (78), and the causative agent has been identified recently in Korean chiggers by Fuller & Jackson (81).

Concrete evidence suggesting the existence of a jungle cycle in scrub typhus was obtained by the pertinent observations of Traub *et al.* (82) in the primary jungle of Malaya. They recovered the causative rickettsiae from the tissues of rats belonging to three species which are found almost exclusively

in primary jungle. Infection was also demonstrated in the chigger species *Euschöngastia audyi* (identified at the time as *E. indica*) taken from three squirrels. These mammalian and acarine hosts of the rickettsiae in the jungle are different from the species which occur in inhabited areas and which are known to be associated with the disease in human beings. The authors state (82, p. 272): "The natural cycle of scrub typhus in the jungle and the explosive local outbreaks of the disease in man following exposure in hyperinfected areas of scrub terrain may prove to have a number of similarities to jungle and urban yellow fever." This interesting problem deserves further careful studies of the sort made by Traub and co-workers.

Korean hemorrhagic fever is believed to be an infectious disease and is thought by some investigators to be vector borne. Characteristics which must be possessed by the vector or vehicle of transmission were presented clearly by Gauld & Craig (84). Epidemiologic evidence pointing toward chiggers as possible vectors was presented by Traub *et al.* (85). Until a suitable susceptible laboratory host for the causative agent can be found, however, the vector role of a particular arthropod species can neither be proved nor disproved.

Control of chiggers.—With respect to control of scrub typhus by vector control methods, the observations of Traub *et al.* (83) demonstrated that the application of dieldrin or aldrin to the surface of the ground provided a practical and efficient means for the disinfection of terrain. Chiggers were reduced in numbers to practically nil during a period of five weeks following application of the insecticide. These chemicals were effective against the vector species under natural conditions during a period of heat and rain-fall in Borneo, hence the results are probably directly applicable to field problems in scrub typhus control.

SARCOPTIFORM MITES

The most important member of this suborder is the itch mite, *Sarcoptes scabiei*. Scabies is mentioned in this review in order to draw the reader's attention to important advances made in Great Britain and Denmark during and after World War II. These include studies of the biology of the mite as observed under the conditions of existence in the skin of man in the course of carefully controlled experiments with human volunteers. A recent summary of this information is that by Busvine (86, pp. 258–71). Interested persons who are not familiar with the outstanding work of K. Mellanby and his co-workers, and of B. Heilesen, should consult the references cited by Busvine (86, pp. 281–83).

ORIBATID MITES

These mites as intermediate hosts of tapeworms were reviewed recently by Allred (87). The worms known to be involved comprise 13 species belonging to two families, Catenotaeniidae and Anaplocephalidae, order Cyclophyllidae. Their known acarine hosts include 40 species of Oribatei. Among

the more familiar worms is *Moniezia expansa*. This cosmopolitan parasite of domestic ruminants may gain access to these mammals when they ingest certain free-living oribatid mites which are common in pastures and which sometimes harbor the worm in its cysticeroid stage. There is apparently little clear evidence of host specificity between particular species of tape-worms and their known acarine intermediate hosts. Examples of the fascinating ecologic complexes which arise are described in the references cited by Allred (87).

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MODERN QUARANTINE PROBLEMS¹

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INTRODUCTION

It is customary to start a review of the quarantine problem with a discussion of the 1912 Plant Quarantine Act. This is probably inappropriate since the act resulted from a build-up of sentiment which started long before the act was passed. In this case it is notable that some states, such as California, had started to enact and enforce quarantines as early as 1881 or 1882, or long before the national act was passed. The history of the various quarantine measures and their philosophy has been discussed ably by McCubbin (8) and will not be repeated here.

Quarantine laws were originated because of the need for the protection of agriculture against pest invasions from outside the state or nation or the protection of one area within a state against invasion from another area. The measures introduced were: (a) quarantines with embargoes against the introduction of certain materials which might carry a pest; (b) inspection intended to detect either embargoed material or the presence of pests on nonembargoed material; or (c) treatment of shipments to destroy unwanted pests. Plant quarantine inspectors handle all three functions.

Early efforts to keep out pests emphasized inspection and quarantines of the embargo type, with treatment playing a minor part because satisfactory treatments were not then available. Inspection was better accepted as a potent measure than it is today since relatively little was known about viruses, nematodes, and other organisms that are difficult to find by inspection. Inspection was likely to be more or less macroscopic, with the hand lens considered a satisfactory accessory. Later the microscope was brought into play, and inspection centers where work could be done more carefully came into being. The complicated virus picture which we know today, in which a virus may be present without symptoms in one plant yet wreak havoc in another plant, was unknown, and inspection for spots or lesions and for insects appeared adequate.

Embargo quarantines were faced with the same pressures with which they are faced today and were considered a last resort, and in order to avoid the stigma of commerciality, certain tenets were accepted which to a degree vitiated their effectiveness. To impose a quarantine, it was held that the insect or disease quarantined against had to be known, and there had to be good scientific basis for suspecting that it could become a plague

¹ The survey of the literature pertaining to this review was completed in August, 1955.

if introduced. It is obvious that this is not the best yardstick since many of our worst invaders were either unknown to us before they were introduced into the United States or were not important pests in their original habitat. Examples of this are numerous, as for instance citrus canker, which is not a serious problem in Japan, was imported from that country into Florida and without eradication could have wrecked the citrus production of that state; Japanese beetle was not recognized as the major pest it has become until it arrived in this country; and the tristeza virus was imported into Argentina, Brazil, Uruguay, Paraguay, California, and Florida before it was even recognized as a disease.

Treatment of imported products is gradually increasing as more efficient methods are available, but treatments are usually aimed at a particular pest and are not over-all remedies, i.e., a treatment for fruit fly eggs and larvae in imported citrus might not destroy the spores of sweet orange scab. Increased emphasis on research for the development of treatments is highly desirable, but too much emphasis must not be placed on known pests because too often it is the unknown pest which does the damage.

Attempting to meet the constantly changing conditions, protective measures have developed gradually in a sort of rear-guard action, but whether this is going to be adequate in the future is questionable. We escaped the war period fairly well though we might reasonably have expected the introduction of many insects and diseases as a result of the rapid movement of men and materials under conditions where quarantine control was exceedingly difficult. It is probable that the fact that most of the transfer was from this country to other countries rather than into this country was a favoring factor. In spite of airplanes coming and going by thousands and always carrying personnel some few of whom could be counted upon to be collectors, we appear to have escaped dangerous invasions except for the oriental fruit fly which was found in Hawaii in 1946. Since the war, however, the problems of pest control have been changing rapidly, and we will attempt here to point out some of the factors which seem to be most inimical to our efforts to keep out agricultural pests.

The discussion which follows is divided into two general categories. The first includes the factors which might perhaps be classified under the heading "Politico-Economic Problems," including the problems presented by improved transportation and increased travel, while the second includes a discussion of modern scientific developments which by increasing our knowledge along certain lines points out the extreme difficulty of keeping certain types of pests out of the country except by fully enforced quarantines of the embargo type. The examples in the latter category will be drawn largely from citrus, this being the field where the writer has had first-hand experience, but there is no reason to believe that parallels do not exist in many other crops, and in some cases equally dangerous examples may be familiar to the reader.

POLITICO-ECONOMIC PROBLEMS

The universal shortage of dollars in the world outside of the United States is at the bottom of some of our pressing difficulties. In order to obtain dollars, countries which need them have only two alternatives outside of obtaining loans, which are intended as only a temporary expedient. One is to export products to the United States, receiving dollar exchange in payment, and the other is to import tourists from the United States, who pay for their lodgings, meals, and entertainment with dollars. The tourist business is highly lucrative in some areas, but many more nations are in a position to export materials to the United States than are in a position to import tourists on a scale extensive enough to supply the dollars they need. The result has been a constantly increasing pressure to export goods or products, often agricultural, to this country, and it is essential to our own export business that they do so in order that they may pay with dollars for their imports from this country. It has been presumed that the way to tie these countries to us as allies is to better the living standards of their peoples by accepting imports from them, and many governmental agencies have had a hand in trying to promote such activities. An example of this is the breaking of quarantine barriers for Mexican oranges, imports being permitted subject to treatment intended to kill any larvae or eggs of the Mexican fruit fly. Since the quarantine order preventing the importation of Mexican oranges was designed to prevent the entrance of the Mexican fruit fly, the modification of this quarantine to accept fruit subject to treatment opened the barriers regardless of other possible pest importations. It is not clear whether any study had been made to make certain that Mexican oranges were free of other diseases and insects which we do not have and which might pass the treatment in a viable condition, though we presume this was done.

Recently there has been great pressure to modify the citrus canker quarantine to permit the importation of Satsuma oranges from Japan. Japan does not have an excess of citrus fruit, producing only 19 or 20 million boxes of citrus fruit to feed a population of 85 million people whereas the United States produces in the neighborhood of 200 million boxes of fruit, which is almost entirely consumed by a population of 160 million, or roughly ten times as much fruit for twice as many people. The economic pressure for dollars in Japan, however, is such that exportation to this country is highly desirable for the purpose of obtaining dollar exchange even though the export market may be less profitable to the grower than the Japanese market and the exportation actually reduces the amount of fruit available to the Japanese people who need it. Understandably, Japan needs dollars to buy machine tools and other items, and in view of our government's desire to maintain her in alignment as an ally, we find many of our own people working hard to promote the importation. On the other hand, the representatives of the Florida citrus industry have worked just as hard to maintain the quarantine. We are not prepared to indicate who is right or who is wrong in this

matter, but it is nevertheless a good example of the situation that has developed.

The politico-economic pressure for freer importation of many products is likely to continue for a long time. Brazil and Spain as well as Mexico and Japan would like to export citrus fruits to the United States in return for dollars, while Argentina could easily plant enough citrus to do likewise if the way were opened to her. Nor is this exclusively a citrus problem. Argentina would like to exchange wheat and corn for dollars, as well as meat, which is now held back by the quarantine against the hoof-and-mouth disease.

The fact that an American industry may be operating on a small margin because of low prices as compared to costs of production and marketing is not a deterring factor inasmuch as production costs in terms of dollars are much lower in many of these countries than they are here, and the need for dollars might easily result in government required exports in order to obtain dollars even if the industry had to be subsidized with the national currency.

Unfortunately, quarantines traditionally have been based on specific things such as fruit flies or the hoof-and-mouth disease, for which there is sound evidence scientifically of possible damage if it becomes established in this country. Under such conditions, if a treatment is developed which will kill the organism in question, there is likely to be immediate pressure for relaxation of the quarantine subject to treatment of the product. Other diseases and insects which may be less known and not included in the quarantine are not always considered. A typical case in citrus is the fruit fly quarantine which keeps out South American citrus. When the treatment for fruit was perfected which killed the eggs and larvae of this insect, there was immediate pressure to relax the quarantine in spite of the fact that there was no evidence that the treatment would prevent the introduction of live spores of sweet orange scab (*Elsinoe australis* B. and J.) which is widespread in Brazil, Argentina, Uruguay, and Paraguay. The establishment of this disease in Florida would almost certainly make it necessary to double the spraying with copper fungicides on oranges, and it probably would cause damage in Louisiana and Texas, although Arizona and California might escape because of their dry climate.

Since these pressures to permit imports are likely to continue, more attention should be given to studying agricultural industries in the areas which want to export to us and to tabulate all of the diseases and insects which might be injurious. In the case of citrus from South America, this would add not only sweet orange scab to the quarantine, but also the black citrus aphid (*Aphis citricidus* Kirk.) and probably some mites and California red scale [*Aonidiella aurantii* (Mask.)], which, as far as we can determine, is not present in Florida. Attention might then be given to setting up broad quarantines, thus eliminating the necessity for repeated hearings.

It is conceivable that at the same time consideration should be given to the adequacy of the national supply of a product, a feature that has not

been present in our thinking up to now. This idea is anathema in the minds of many quarantine officials since it seems to smack of the commercial aspect, but in view of our past experience, it seems logical that tighter control is indicated where adequate supplies are produced at a low level of profit within the country than where there is only a small production which is not adequate for the national use.

Another manifestation of this situation is the pressure for baggage examination at the source and for only a spot check of baggage. The purpose is of course to reduce the annoyance to tired travelers at the end of a long trip. From the standpoint of Customs, which probably collects little taxes from routine baggage inspection, these changes may not be of particular importance, but from a crop protection standpoint, it is reasonable to assume two things, first, that inspection at the point of origin will place the inspection where there is less local pressure to do a careful job and less personal interest, and, secondly, it will eliminate interceptions by the percentage that the inspection is reduced. Some checks have indicated, however, that the percentage of interceptions may be decreased more than the percentage of bags inspected is decreased. Since the Plant Quarantine officials at the ports must depend on the Customs and are called upon only when the Customs finds plant material, the effectiveness of the inspection depends upon the Customs fundamentally, and a modification of Customs procedure is bound to affect the quarantine inspection.

Danger of entry of dangerous material is from two sources: commercial shipments and travelers. Prior to World War II, the commercial shipments probably offered the greater hazard, but today the writer would venture the opinion that travelers constitute far more hazard than commercial shipments since the latter can be better controlled by the use of permits and inspection plus treatment where indicated. The increase in speed of travel needs little comment. From New Orleans to Buenos Aires or Rio de Janeiro formerly required 16 to 19 days by the faster steamers, but now they are both closer than 24 hours by plane, and Europe is even closer. Essentially this means that a stick of bud wood or a cutting can be carried in the pocket with even elementary protection and arrive in viable condition. In the days of leisurely boat travel, much propagating material lost its viability during the passage and the more leisurely inspection of baggage at the port of entry probably detected more contraband. Air travel, however, has a much more hurried atmosphere, and passengers resent a detailed and slow-moving inspection, especially if it comes at the end of a long, tiring trip. It is this atmosphere which pleads for reduced baggage inspection and point-of-origin inspection, and all this tends to increase the hazard. Within the country, similar plane service plus fast auto travel and thousands of travelers pose the same problems. California may inspect automobiles at its border, but it would be difficult to exercise control over the thousands of plane passengers who can cross the continent in a day.

While only a few travelers carry dangerous material, there is still the ques-

tion as to why this limited number do so. Traveling across country, the carrying of edible fruit for refreshment is understandable, but the carrying of seeds, bud wood, and cuttings is in another category. The writer's observation is that most of the propagating material is carried by amateurs and not by professional nurserymen or propagators. Seeing an interesting fruit or flower in the course of travel frequently gives the amateur gardener or horticulturist the desire to bring it back to his own home and propagate it for its curiosity. Many such collectors carry the material in ignorance of the rules and the damage that might be done, but some do so with the knowledge that it is illegal. Many travelers look on the inspection as a sort of sporting proposition; the passing of material by the inspectors is seen in the same light as avoiding the traffic policeman. Dick (5), summarizing for 1952 the border inspection carried on by California, reported that $3\frac{1}{2}$ million vehicles carrying 9 million passengers were inspected, and 87,592 lots of plant material were intercepted. The passengers were given the opportunity to declare material after an explanation of the rules, but even with this explanation 11,088 undeclared lots of material were found on inspection, and it is highly probable that other lots escaped detection, being too well concealed or being carried on the person of the traveler.

An excellent example of the effects of improvement in roads and transportation has been the transportation of Mexican fruit fly to the west coast of that country and up to the California border since good roads in that area have made possible rapid transportation. This area is free of native hosts and the evidence seems conclusive that it has been carried by shipments of fruit or by fruits carried in cars (2). The eventual completion of the Pan-American Highway will compound the problem since its opening will start a flood of cars going and coming from Panama to the Texas or California border. Recently the Mediterranean fruit fly has been found in Costa Rica by Mowry, and with airplane flights from that country direct to Florida and by stages to California and Texas, it is going to be difficult to keep it out of this country. The problem will be much worse once automobile traffic is established through the finishing of the gaps in the Pan-American Highway. It was very obvious during our eradication campaign in Florida that our most active agent in spreading the Mediterranean fruit fly was automobile travel, with infested fruit being tossed out when travelers who had bought it for refreshment found that it was spoiled.

The carriage of insects under these conditions is not limited to those things deliberately carried by man, since the insects themselves may ride on planes and in automobiles, and the introduction of the air-conditioned automobile will probably increase the hazard since the windows will usually be kept closed between stops. While sprays for the disinfection of the interiors of vehicles are being improved, there is always the possibility of insects in crevices beyond their reach and only accessible to actual fumigation. Nor are fungi to be omitted from this discussion, since their wind blown spores may easily travel by either car or plane.

Traffic will increase, not decrease, unless a war or depression interferes, and means must be found to meet this situation. Random sampling of luggage may be satisfactory from a Customs standpoint, but from a quarantine standpoint it is a step backward. Better education of travelers would improve the situation, providing the education is attempted in an entertaining and striking manner; bureaucratic verbiage will not suffice nor will education take care of the man who regards passing things through the inspection as a sporting hazard. Educational literature will have to be furnished foreign visitors as well as Americans if it is to be effective. While work on treatments is of utmost importance for commercial shipments, it is to no avail on smuggled material. In the end, it is probably going to be necessary to greatly increase the inspection force and to set higher standards for the inspection of vehicles and their treatment if we are to remain relatively free of invaders.

SCIENTIFIC CONSIDERATIONS

Scientific developments of recent years and the expansion of knowledge concerning diseases and insects have brought to light some types of pests which are not readily detected by any available inspection procedures, nor are they controllable by available treatments. Inspection has already been brought to the level of the microscope instead of the hand lens, and effective treatments for many things have been developed, but microscopes will not serve to identify a virus in a plant which does not exhibit symptoms but may serve as a carrier. Information on nematodes and some recent disastrous experiences have indicated that we know very little about this type of pest and while delaying to acquire knowledge, we may very readily come to grief. Citrus presents two very good examples of the problems involved in these two fields, and they will be reviewed to show the potentialities of the situation, but it should be remembered that this sort of difficulty is not confined to citrus, and these are only examples. Undoubtedly similar examples could be quoted for many other crops on the basis of present knowledge, and still other examples exist of which we have no present knowledge.

VIRUS DISEASES

Virus diseases have always presented a quarantine problem [Orton (14)], since symptoms frequently are difficult to identify at certain stages of growth, and the virus itself cannot be seen with the compound microscope. Moreover, virus diseases undoubtedly exist undetected in many agricultural areas where scientific work has not been intensive. As knowledge of viruses has increased, the magnitude of the problem has been further revealed. The tristeza disease of citrus is an excellent example of a virus disease which has been transmitted to several new areas before being recognized as a disease. A brief review of its complex manifestations will serve to point up the extreme difficulty of setting up and maintaining effective protective measures short of embargo of all propagating material.

The tristeza or quick decline disease of citrus is now known to be attributable to a virus transmitted with great efficiency by the black citrus aphid, *Aphis citricidus* Kirk., and very inefficiently by some other aphids. When sweet orange, mandarin, or grapefruit trees budded on sour orange, grapefruit, or commercial lemon become infected with a virulent strain of the virus, they are killed in a relatively short time, or in the case of a mild strain of the virus permanently stunted, whereas if budded on sweet orange, mandarin, rough lemon, or *Poncirus trifoliata*, there are no obvious symptoms and to the eye the trees appear healthy. Certain secondary symptoms may occur, such as stem-pitting, dwarfing of the tree and under-sized fruit, but these, at least in the early stages, are not obvious symptoms. Such trees are effective carriers of the virus which may be readily transmitted by budding or grafting or by the black citrus aphid.

This disease has probably been present in South Africa since the inception of the citrus industry in that area, but was not recognized as a disease until 1946 by Meneghini (10, 11) in Brazil and Wallace & Fawcett (18) in California. The failure of sour orange as a rootstock in South Africa has been interpreted as a lack of adaptability to the soils of that region. From South Africa, the virus was probably carried to Australia in nursery stock or bud wood and in 1928 was imported into Argentina in nursery stock budded on rough lemon stock, which appeared to be healthy. Wherever this stock was planted, trees budded on sour orange, which was the common and recommended stock, started to die with the ultimate loss of trees running into millions. About 1937 it appeared in Brazil with devastating results to the Brazilian citrus industry, which also had depended heavily upon sour orange stock. While it may be argued that the importation of infected nursery stock was a piece of inexcusable carelessness, the case is not tenable under the time honored tenets of plant protection. South Africa had a well established citrus industry, and no important disease or insect was recognized that was not already present in Argentina. The nursery stock being budded on a tolerant stock appeared healthy on inspection. While we now know certain secondary symptoms that might have been detected, there was nothing known at that time which would point suspicion at the nursery stock. Whether the black citrus aphid was brought with the same stock is not clear, but even if it was, its potential as a pest was not known.

The time and method of the entrance of the virus into California and Florida is not known with certainty, but Olson & Sleeth (13) have recently reported evidence that it quite possibly was brought to Texas with the Meyer lemon, which had been introduced from Manchuria by the plant explorer, Dr. Meyer. Wallace & Drake (17) have also shown that the Meyer lemon was probably one of the early imports of the virus into California. This importation was handled with due regard to the knowledge then available, but like the Argentina importation, was disastrous, though not to the extent that it was in Argentina or Brazil because no highly effective vector was present. It is quite possible that the virus came to Florida also through the same

channel, since it has obviously been present there for many years. As in California, it was not brought forcibly to the attention of technical workers at an early date because of the inefficiency of the native vectors.

That this is not an isolated instance is testified to by the fact that the work on tristeza has helped to clarify the case for two other citrus viruses of somewhat similar characteristics, xyloporosis and exocortis. The former produces damaging effects when trees are budded on the so-called sweet lime, the latter when trees are budded on *P. trifoliata*. Both of these viruses are apparently present in most of the older varieties of oranges but only produce damaging symptoms when attempts are made to use sweet lime or *P. trifoliata* as stocks, as was the case in Argentina following the destruction wrought by tristeza when both stocks were tried extensively for late oranges, with disastrous results. Since infected scions on either of these stocks usually grow normally for five to seven years after budding, both of these are particularly insidious. Recently groves budded on Rangpur lime in the state of São Paulo, Brazil, are reported by Moreira (12) as suffering symptoms at about five years of age, similar to those exhibited by trees infected with exocortis, and it may be the same or a different virus.

It is reasonable to assume that other viruses of similar characteristics exist in citrus and also in other crops, many of them yet unknown to scientists. These together with the viruses which are damaging to one host but show little symptoms in another plant species which may act as a carrier represent an extremely difficult problem. In certain phases they are impossible to detect by inspection, and treatment of propagating material to destroy viruses has as yet shown only limited application [Orton (14)]. The only sure protection in such cases appears to be the embargo type of quarantine, though some relief might be obtained for the introduction of new varieties by permitting the importation of only seeds, which procedure would have the virtue of straining out many of the viruses though not all.

The problem of symptomless carriers is present in many crops. Chickasaw plum may be a symptomless carrier of peach mosaic, according to Hutchins *et al.* (6), while Kunkel *et al.* (7) and Manns *et al.* (9) report that peach yellows and little peach have the Myrobalan plum (*Prunus cerasifera*) as a symptomless carrier, and several varieties of the Japanese plum (*Prunus salicina*) are symptomless carriers of little peach. Dodder (*Cuscuta californica*) is reported by Bennett (3) as a symptomless carrier of a virus causing damage to sugar beets, cantaloupes, tomatoes, and several other crops.

In addition to the problem of symptomless carriers, there is always the possibility of the presence of a virus but the absence of a highly effective vector. This is the case with tristeza in both Florida and California and apparently has been for many years. The introduction of the black citrus aphid (*Aphis citricidus* Kirk.) could change the picture in a very short time since it has been found to thrive in both a humid climate similar to that of Florida in northeastern Argentina and eastern Brazil and an arid climate in western

Argentina and in Peru. Only a few years ago the introduction of this aphid could have created disaster, in the citrus industries of California, Florida, or Texas though the presence of a dangerous virus was unknown and the aphid's role in disseminating it equally unknown.

THE NEMATODE PROBLEM

In recent years nematodes have been receiving increased attention as plant parasites. The introduction of the golden nematode was a severe blow to the Long Island potato growers, and the measures used to limit its spread have been adequately reviewed by McCubbin (8). In the late 20's in Florida, a decline of citrus trees was noted which has become steadily more serious as time has progressed. It was not until 1953 that Suit & DuCharme (16) reported that the burrowing nematode [*Radopholus similis* (Cobb) Thorne] was the primary causal agent. This was the first time that this nematode had been reported as a parasite of citrus and only the second time it had been reported from the continental United States, the first report being by Rands in 1929 (15) from sugar cane in Louisiana, from which area it has not been collected since that time. Further studies by Brooks (4) and others have shown that it is a parasite on many tropical plants in Florida and that in many cases little or nothing in the way of damaging symptoms is visible though it will put citrus out of commercial production.

Early studies on this disease had shown that it started most often in citrus trees around houses and buildings that had been landscaped and that probably ornamental yard plants were carriers. This now seems well confirmed, and control of ornamentals in nurseries will be as vital as the control of citrus nursery stock. There is every reason to believe that this nematode is an importation brought in on tropical plants; in fact, quarantine inspectors in both Florida and California are now intercepting many infested shipments from such areas as Hawaii, Puerto Rico, and South America.

Here again we have the example of a pest that became established before anything was known concerning its potentialities, which are evidenced today by some 3,000 acres of declining citrus grove plantings in Florida while it is still unreported on citrus elsewhere. In the process of the research work which led to the identification of the burrowing nematode as the cause of spreading decline, several other nematodes hitherto unreported on citrus were found, which merely serves to emphasize our lack of knowledge concerning nematodes as potential crop enemies.

While the golden nematode was known to be a dangerous parasite of potatoes in Europe before its introduction into this country, the burrowing nematode had no such history, and yet it is potentially as dangerous as the Mediterranean fruit fly as far as the citrus industry of Florida is concerned, and there is no reason to feel that it is an isolated example.

GENERAL DISCUSSION

High speed transportation and increased travel have brought the quar-

antime problem to the point where decisions must be made or we may easily face disaster. One of our obvious weaknesses is our lack of information concerning pests existing in many foreign countries from which we receive imports. In our effort to help others by sending agricultural experts to many foreign countries, we could perhaps do ourselves a great deal of good by sending men capable of making a detailed study of the pests existing in various countries and cataloging them with reference to their potentialities. Such studies would be valuable to us in our protective efforts and would probably be equally profitable to the country where they are made, and even detailed research would be justifiable in the interests of both parties.

A review of our philosophy of quarantines seems inevitable. The concluding statement by Annand (1) that quarantines must be biologically sound seems untenable if by that you mean that you must have sound, known evidence that the pest you are quarantining against is recognized by scientific authorities as dangerous to our own agriculture. The case of *tristeza* in Argentina and Brazil and the burrowing nematode in Florida are mere samples of what unknown pests can do to an industry, and we have had similar examples in other crops. It seems inevitable that we must go beyond the bounds of known information and give some broad consideration where crops are sufficient for our needs, to quarantines which cover all of the possible pests of a crop. A broadening of our thinking plus increased studies in foreign countries to develop information concerning our probable enemies may readily prove the most important factors in a very difficult situation. In view of the potentialities of the situation, the importance of control research in order to have information ready when we receive an invader seems greater than ever.

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THE FUNDAMENTAL THEORY OF NATURAL AND BIOLOGICAL CONTROL¹

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By biological control we mean the control of an animal or plant species through the action of other living organisms, as opposed to physical non-living agents. By control, the economic entomologist usually means the existence of a condition where the population of an injurious insect is below the numerical level at which it begins to cause measurable economic damage. Artificial control is attributable to human intervention; natural control, to the nonhuman agencies acting in nature.

The expression "natural control" in a broad sense refers to the fact that no organism multiplies indefinitely. If the meaning of the term is not carefully restricted, so that it corresponds only to what is objectively evident in nature, the inquiry about natural control may be prejudiced from the start. If we say simply that natural control refers to the fact that no organism multiplies indefinitely, we are stating an undeniable objective fact without any danger of slipping at our first step from facts into theories.

Natural control, then, refers to the fact that no organism increases indefinitely in number without limit. So far as our experience goes, this is true not merely of species attacked by parasitic and predacious organisms but of all species. Biological control, by which we mean the control of organisms by other living organisms, is therefore only one aspect of natural control, and it is within the framework of the general theory of natural control that the special theory of biological control must be constructed. The problem of natural control is itself only a subdivision of the field of general ecology which considers the relation between an organism or a population of organisms and the environment. So much has been written on this vast subject that an adequate summary could not possibly be given within the compass of this article. Useful recent summaries have been given by Solomon (16), Ulyett (25), Glen (8), and Andrewartha & Birch (1). This discussion, therefore, will be limited mainly to the important essay by Nicholson entitled "An outline of the dynamics of animal populations" (12). In this essay Nicholson develops in various ways the views he expressed in an earlier paper, "The balance of animal populations" (11), defends them against criticisms, and attempts to support them by experimental evidence.

Although his views have been criticised on several occasions, Nicholson does not seem to have modified them in any important respect. He has not done so because the method he has adopted confers, in a sense, a certain immunity to criticism. He says,

The foregoing discourse begins with the statement of certain facts of common

¹ The survey of the literature pertaining to this review was completed in June, 1955.

knowledge which are so well established as to be incontrovertible. The implications of these facts are examined in relation first to very simple situations, and then to progressively more complex ones and eventually general conclusions are reached concerning population, growth and maintenance under natural conditions. Although much use is made throughout of further evidence derived from observations and experiments, the method of investigation is essentially deductive reasoning. The approach is thus the same as that used in earlier articles. . . .

The significance of these remarks will become clear to us if we consider the difference between the deductive and the inductive sciences. In the inductive sciences of which philosophy and mathematics are the principal types we begin with a general principle or concept which exists, as such, only in the mind. From this concept we proceed to certain conclusions, not explicitly evident in it, by pure reasoning. It is quite otherwise with regard to the objects of the inductive or experimental sciences. These sciences, of which biology is one, cannot build themselves up by pure reasoning, involving connections seen to be certain; they have to develop by the accumulation of observations, and though by repeated observations we can attain a very high degree of probability we cannot reach perfect certainty as we can in a science like mathematics.

A deductive science, if correctly constructed, forms a solid block without a fissure. If the first principles are true and the reasoning is accurate, the conclusions must be correct. There is no real need to import observational data. For this reason the attitude of the convinced adherents of a mathematical deductive biology is very different from the attitude of workers who use the deductive method, either philosophical or mathematical, only in an incidental and casual way, either as an introduction to the subject or as a stimulus and guide to observation and experiment. For the convinced exponent of the deductive method the data of observation and experiment are really not absolutely necessary. They merely confirm what he already knew, and their main value is probably the persuasion of recalcitrant or obtuse opponents.

On the other hand, for the worker who uses the mathematico-deductive method only incidentally, the data provided by observation are of vital importance. He recognizes that they may oblige him at any moment to recast his mathematical formulation. This is not, as Nicholson appears to suggest in one section of his paper (12, p. 54), because he is less devoted to "thought" than the exponent of the deductive method, but rather because he recognizes the true nature of biological science and is constantly and keenly aware of the complexity of natural phenomena.

In discussing Nicholson's papers (especially 11) I expressed the view (23) that the numerous conclusions he reached in regard to population problems were the result of arithmetical calculations expressed in nonmathematical language. Nicholson has not rejected this view and indeed the quotation given above appears to confirm it, since Nicholson says that the implications of his basic thesis are examined in relation first to very simple situations and

then to progressively more complex ones, so that eventually general conclusions are reached concerning population growth and maintenance under natural conditions. It does really appear, therefore, that although the results of observation may be used to illustrate and to convince the doubters, they are not really needed to give us information about what is happening in nature. This can be deduced with incontrovertible certainty from the incontrovertible premises adopted. If these claims are correct it is certainly to Nicholson's theory of natural control that students of biological control should turn for guidance in their research work and an understanding of their problems. It is therefore important to examine the premises and procedures connected with the theory in detail. Since my criticisms are based on my own views, I shall have to quote at some length from my own published papers in this field.

In his 1954 paper Nicholson (12, p. 59) lists a number of considerations which appear to him to be axiomatic and therefore provide a solid basis for an understanding of what he calls population dynamics. These are as follows: (a) All animals have an innate ability to reproduce and to multiply under favourable conditions. (b) The favourability or otherwise of the environment for a given species determines whether its population is permitted to grow or is caused to decrease. (c) As every animal born must die and can die only once a population cannot persist for long periods in any given environment unless the birth rate and death rate are virtually equal (when each is averaged over a representative period); for if numbers of births and deaths remain appreciably different the population would decrease or increase geometrically so either directly falling to extinction or causing its own extinction by overwhelming its environment in a comparatively short time. (d) As populations grow, the constituent animals use up more and more of the limited available quantities of depletable requisites (such as food and favourable space), and increasing density often intensifies the action of inimical factors (for example by increasing the densities of any natural enemies dependent upon the animals concerned or by increasing the concentration of harmful metabolites). (e) Because animals produce such an effect upon their environments, growing populations progressively reduce the favourability of some factors whereas decreasing populations permit favourability to recover. Such compensatory reaction inevitably governs population densities at levels related to properties of the animals and those of their environment. (f) Consequently, when operating in association with density governing factors, nonreactive factors (such as climate) may have a profound influence upon density, for many nonreactive factors influence either the properties of the animals or environmental favourability. (g) Operating by themselves, however, nonreactive factors cannot determine population densities for, if sufficiently favourable, they permit indefinite multiplication or, if not, they cause populations to dwindle to extinction. On the other hand they inevitably limit distribution to those areas within which they are favourable.

In order to define the areas of agreement and disagreement I may compare

the remarks made in my paper on "Natural Control" (21, Section VII, p. 275) published in 1929.

When conditions in a locality inhabited by a given species approach the optimum the species automatically increases in numbers. This increase constitutes what we call technically, an outbreak, which is of necessity, as we have already said, an abnormal phenomena. The organism considered may continue to increase in numbers for a considerable time but it obviously cannot go on increasing indefinitely. As it increases in numbers it necessarily spreads both in space and in time. As it spreads it moves to points outside its optimum environment when its rate of multiplication immediately diminishes. Furthermore as the population of the species becomes more numerous the action it exerts upon its environment both indirectly and directly, increases in intensity, producing changes which are, on the whole, in so far as they depend purely and simply on the numerical increase, disadvantageous, since they consist to a great extent in the progressive exhaustion of the nutritive power of the environment in relation to the species considered. It must also be remembered that the condition of the globe at any point is never constant. A region which today presents conditions optimum for a given species may depart from optimum conditions in the direction X one year and in the direction Y in the year following.

Thus under even most favourable conditions there cannot be a continuous and uninterrupted increase in numbers but simply an oscillating movement whose amplitude will vary according to circumstances but is more likely to be feeble than extensive because of the narrowly circumscribed optimum and restrictive adaptive powers of the majority of species.

Whether the change in numbers of the species will take the form of an oscillating movement about a mean value depends, however, on chance. Some species go on increasing for long period of years. Others in consequence of environmental changes producing conditions with which they cannot cope, decrease in numbers and disappear completely. Numberless catastrophes of this kind have occurred in the past and though the majority of them escape notice, still continue to occur. The only reason why oscillation about a mean population value is on the whole more frequent than a steady increase or decrease, is that on account of the great complexity of the natural environment, changes unfavourable to a species do not usually occur simultaneously in all the localities it inhabits so that although its numbers diminish at one point they increase at another.

In the bulletin "Some Beneficial Insects" published by the British Ministry of Agriculture and Fisheries in 1939 (22) it was noted that not only do organisms exhaust the available supply of food, but this is replaced by their waste products.

In section X of the 1929 paper (21, p. 278) there are some additional remarks about the action of biotic factors: parasites and predators. I said,

It must also be noted that one of the most important factors in determining the status of the parasites and predators is the relative abundance of the food supply, i.e. of their hosts. The more perfectly uniform and continuous the distribution of the hosts, the more likely are the parasites to flourish. Furthermore, since the essential characteristic of the parasite or predator is that it increases at the expense of its host the regions in which the host is abundant are those in which it is likely to be most effective as a controlling factor.

Comparing the propositions enunciated by Nicholson in 1954 with the views I expressed 25 years earlier we find I think that up to and including Nicholson's proposition four, there is substantial agreement. This applies also to the first sentence in his proposition five. From this point onward there is a subtle but very important divergence of views. This is because the agreement does not concern propositions that are really fundamental.

My view expressed clearly in my 1929 paper (21) is that the natural control of organisms is primarily attributable not to any complex cosmic mechanisms or regulatory factors but rather to the intrinsic limitations of the organisms themselves. Every organism, I said, has specific characteristics that vary about a mean but may be considered for practical purposes as fixed. These specific characteristics imply and indeed include specific needs. An environment which meets these specific needs is, for a given species, the optimum environment. Given this optimum environment, indefinite increase at a specific rate is possible. But the environmental conditions of the globe vary from point to point and are probably in no two places exactly alike. From this it follows that at a given moment, in a given area, the precise environmental complex constituting the optimum for a given species will be found at relatively few points. This is the real reason that organisms and, in particular, injurious insects, do not often increase to the point where they are devastating plagues. The fundamental constitution of the universe, composed of a multitude of specifically different and interacting things, living and nonliving, necessarily implies a limited possibility of existence for any particular one of them. The fact that there are species, that is, a variety of essentially limited things, means that their ability to increase is necessarily limited. From this it follows, that the causes of natural control, even for a particular species, are essentially multiple and variable: difficult therefore to bring within the compass of any simple definition. When we recognize the specific intrinsic limitations of organisms we have, in my opinion, arrived at the absolutely fundamental explanation of natural control: that is to say, the fundamental explanation of the fact that no organism increases without limit. This is a philosophical explanation which, indeed, verges on the metaphysical, and this I think is a sign that we have really got to the bottom of the subject.

When we first look at the world it appears to us as a chaos. What we initially grasp, says Aristotle, in the first book of the *Physics*, "is rather confused masses, the elements and principles of which become known to us later by analysis." But he also says that when we follow this method, we are starting from the things which are more knowable and obvious to us and proceeding "toward those which are clearer and more knowable by nature." He means that as we investigate the apparent chaos we find that it is made up of elements which are definable, therefore definite and therefore intelligible. These elements are the specific entities of which the world is composed. However, between these elements there is a stupendously complex system of changing interrelations. Undoubtedly a certain order can be discerned, which

we may define by saying that we can recognize an interdependence between the elements or beings of which the world is composed. Plants develop at the expense of the inorganic world. Animals subsist on plants or on each other. The entities we define and catalogue behave in certain definite ways following laws rooted in their specific natures. To this extent their behaviour is predictable and intelligible. But this cannot be said of all the "contacts" that occur between these entities. Here we have what Carnot called the intersection of independent causal series giving rise to what we ordinarily term "accidents." Objectively considered, the world is therefore a mixture of law and lawlessness, of order and disorder, of necessity and contingency, of intentional and random action. For this reason any individual entity cannot always or indeed often follow its predestined path and realize all its potentialities. The more venturesome it is, so to speak, the more likely it is that it will eventually be caught in the destructive web of interrelations which exists because the world is made up of a multitude of specifically different things. But though we find in these considerations a general explanation of the fact that organisms do not multiply without limit we are led also to recognize that we are not likely to discover any simple formula or definition giving us any more specific cause for "natural control" in the sense defined above. We might in fact compare "maintenance factors" and "controlling factors" to truth and error. Truth is simple but error is multiple; the conditions for the existence of a specific organism are definable, but any departure in any direction from these conditions will or may cause its extinction.

With these views we must now compare and contrast the explanation of natural control given by Nicholson.

As Nicholson has said his method is primarily deductive. Nevertheless it is based upon an induction or in other words it starts with a proposition which expresses, in the opinion of Nicholson, a natural fact. This proposition is that populations exist in a state of balance. In the discussion of Nicholson's views published in 1939 (see 23) I expressed the opinion that this was his fundamental thesis. He has not objected to this view, and his most recent paper indicates clearly that it is correct.

Most of the authors who have discussed Nicholson's work seem to have had some difficulty with his concept of balance. This is evident, for example, in the discussions of Solomon (16, see particularly section D. 2., pp. 26 and 27), and of Glen (8, pp. 399-400). For this confusion I think Nicholson himself is at least partly to blame. In his 1933 paper (11) he endeavoured to give a rigorous demonstration that populations always exist in a state of balance. That this is so, he said, is clearly evident from the fact that the population density of a given species at a given moment and in a given place has a certain definite or determinate value. Some reason why this value is what it is must exist; it must have a cause or complex of causes whose action is exactly what is required to bring it to the point it has attained. Animal populations

must therefore "exist in a state of balance, for they are otherwise inexplicable." To make this evident Nicholson took the analogy of a balloon.

A balloon rises until the weight of air displaced exactly balances the weight of the balloon but if ballast be then discarded the balloon again rises until it reaches a new position of balance. Because a balloon in the air is a balanced system there is a relation between the weight it carries and the height it reaches. Without balance the height reached would be indeterminate.

Similarly, without balance

the population densities of animals would be indeterminate and so could not bear a relation to anything.

As I pointed out in my 1939 paper (23) the statement of Nicholson is in a sense quite acceptable. It is true that if population densities are indeterminate then they have no definite value. Therefore they have no definite cause or are undetermined. But every effect that exists has had a cause proportionate to it. Population densities, since they are definite, must therefore have a cause proportionate to their actual value at any time and place. From this argument Nicholson concluded that balance exists. It is clear that by balance he simply means the cause-effect relation. If we agree that this is what is meant by balance then it is indeed evident that balance exists and that populations exist in a state of balance. In this argument the concept "in the state of balance" is the exact equivalent of the concept "caused." Nicholson is therefore simply saying that populations are what they are because things are what they are. He is saying something that is undoubtedly true, though the truth is hardly worthy to be termed more than a truism or even a platitude. However, the simple fact that populations are what they are, because things are what they are, was not by any means what Nicholson intended to demonstrate. He intended to demonstrate the existence of balance as he defines it in his theory of populations. Solomon, taking the stand that the word equilibrium is synonymous with balance, said (16, p. 26) that the population should be regarded as approaching balance in so far as it approximates to a steady density. This was the view expressed by Elton (5, 6), Chapman (1a, 2), and Lotka (10).

These views are emphatically rejected by Nicholson. He is of course entitled to use the word balance in any way he wishes provided he defines his meaning. In his 1954 paper (12) he makes several efforts to do this and (p. 50) says

that the balance a population maintains, in spite of the varying conditions in its environment can be likened to that of an equestrienne dancing on the back of a horse, cantering around a circus ring; her movement is partly due to her own action and partly to that of the horse, and she remains poised upon the horse's back, by expertly adjusted compensatory reactions to all the disturbances which tend to cause her to fall.

On page 23 he says

that the ordinary usage of the word "balance" implies controlled movement, or at least potential movement, about a position of true equilibrium. Think for instance, he continues,

about the balancing feats one sees on the stage or at a circus: always the mechanism is expertly controlled corrective movement which opposes each departure from the position of equilibrium. *Balance* refers to the state of a system capable of effective compensatory reaction to the disturbing forces which operate upon it, such reaction maintaining the system in being. It is the exceedingly important state of compensatory reaction upon which populations, in common with ships on stormy seas, depend for their continued existence.

In the opening summary of his work Nicholson says, that "governing reaction induced by density change holds populations in a state of balance in their environments. The characteristic of balance is sustained and effective compensatory reaction. . . ."

I have assembled all the important references to the idea of balance I have been able to find in Nicholson's essay. I do not think they are as lucid as might be desired. I do not think the introduction of tight-rope artists, bare-back riders, and ships on stormy seas help us to get an objective view of the population problem though they perhaps help us to understand how Nicholson is thinking about it.

The first point to which I must refer is that the various cases cited are not really comparable. The balloon and the ship are purely passive entities. The balloon, having certain physical characteristics, rises to a certain height in the air. It is actually forced to this height by air pressure. If ballast is thrown out it rises until the point where its weight is equal to the weight of air it displaces. The ship on the stormy seas is just like a cork. Because of its physical properties it floats and although it moves up and down with the movement of the waves it usually does not turn over because it has been constructed so as to remain right side up. The bare-back rider and the tight-rope artist are quite different. Here we have entities which are actively adapting themselves by compensatory movements they originate, so that they can remain in the positions they have selected. It is a little difficult to see what can be gained by applying the same word to phenomena that are so radically different. However the case of the balloon as we have seen, had an important place in Nicholson's fundamental argument, and the transition from the balloon to the population is somewhat facilitated by the introduction of the bare-back rider and the tight-rope walker. If we say that we know that they are in a state of balance because their position always has a determinate value, then we can go on to note that this value is maintained by a compensatory reaction: by controlled movement, which if it does not actually exist at every moment at least exists "potentially."

I cannot see that these arguments have any value as proofs that populations are always in a state of balance, unless we simply mean that they are

what they are because things are what they are, a concession which I am sure would be quite unsatisfying to Nicholson.

However we can see, in a general way, what Nicholson is driving at. To be in a state of balance in his sense a population need not be stationary. It may be stationary, but it may also be moving. Though he says that populations exist in a state of balance it seems from the analogy of the tight-rope walker that they might at a particular instant be out of balance; but if they are, they are already taking steps to reach a state of balance. The term balance, he says, implies not only controlled movement but potential movement about a position of true equilibrium. The words potential movement do not indicate the real movement but merely the real capacity to execute the movement. The power to execute the movement need not be exercised as long as the system is in a position of true equilibrium; but it is there ready to be exercised if the position of equilibrium is lost. From the analogy of the bare-back rider it is clear that equilibrium may be maintained at different levels because the bare-back rider is bouncing up and down. Nevertheless no matter at what height from the ground her feet happen to be she is still capable of the movements required to restore her equilibrium and will execute these if the necessity arises.

We have now, I think, grasped Nicholson's concept of balance accurately enough to explain it to others in a way Nicholson himself would consider satisfactory. Nevertheless I do not think we can agree that "balance" is an appropriate word to designate this concept. It is clear from the preceding quotations that Nicholson uses it in a way that deprives it of the precision necessary for a scientific term. I agree with Solomon (16) that Nicholson's use of the term seems quite inappropriate and is bound to create confusion. Furthermore the argument based on the analogy of the balloon to prove the existence of balance in populations is valueless because the word "balance" is not used in the same sense in the two cases.

If then we discard the word "balance" as an unsuitable term for the state of populations, as described by Nicholson, what can we substitute for it? The most appropriate word seems to be "regulation." This certainly applies to the tight-rope walker, who adjusts or regulates his position, making it conform to the rule he has adopted, which is to remain on the tight-rope. But on closer examination we find this term a little hard to retain. If we say that the thermostat "regulates" the room temperature we mean that it keeps it at a constant level. The height of the balloon in Nicholson's example, is not "regulated" in this sense; it merely "varies." Nicholson does not claim that populations are regulated in the strict sense. Such regulation as there is, allows them to oscillate around a "position of equilibrium" where they do not necessarily ever come to rest. Furthermore, this imaginary position of equilibrium is constantly changing, like the position of the balloon though not quite like the position of the tight-rope artist or even of the ship, which move within much narrower limits.

The concept indicated by the word "regulation" is fundamental to the

theory of Nicholson. To grasp his meaning we must, I think, refer not to balloons, ships on stormy seas, or even natural populations but rather to the mathematical model which represents this concept: the Lotka-Volterra equations and particularly those indicating the variation in the population of a prey species and of the predator or parasite that lives at its expense. These equations are now familiar, but a brief explanation of their construction is necessary for reference.

Suppose that the reproductive rate of the prey in the absence of the parasite is s . The death-rate of the predator in the absence of the prey, will be negative and maybe written as $-d$. We may now say that s diminishes in proportion to p (population of the predator) and to the extent $k_1 p$, while $-d$ will fall proportionately to n (population of the prey) since the more prey there are the more slowly the predator will die off. This change may be written as $k_2 n$. The rates of increase of the two species will then be

$$\frac{dN}{dt} = (s - k_1 p)n$$

$$\frac{dP}{dt} = (k_2 n - d)p.$$

The factors k_1 and k_2 are coefficients of defence and attack, which correspond to the particular attributes of the two species concerned.

These are differential equations, representing the course of events when increments and decrements of populations and time (n, p, t) are infinitesimal; that is they correspond to a continuous process or flow. Therefore any change in the host population is instantly reflected in the parasite population and vice versa. The population values rise and fall in a series of cycles, repeated *ad infinitum*. The average values of the two populations for a period are respectively,

$$n = \frac{d}{k_2}, \quad p = \frac{s}{k_1}$$

so that they depend on the specific properties of the parasite and the prey, not on their initial numbers. We could, however, postulate that these properties change with environmental conditions, in which case the average values of the populations would also change. We would thus have oscillations about a changing position of equilibrium. It is clear that the Lotka-Volterra model corresponds so closely to Nicholson's concept of equilibrium or balance that it may be considered the basis of his theory of population changes. The same fundamental idea is expressed in the Verhulst equation, which may be written

$$\frac{dn}{dt} = (s - k_1 n)n$$

and represents the growth of a population at the expense of its food supply, which is considered as passive and progressively depleted, though in this

case the population tends toward a stable condition. Nicholson says, indeed, and not without reason (12, p. 31) that the Lotka-Volterra equations have only a limited application, but he admits (12, p. 41) that his primary assumptions are the same as those of Volterra. In the first conclusion given in his summary (12, p. 10) he says:

Populations are self-governing systems. They regulate their densities in relation to their own properties and those of their environments. This they do by depleting and impairing essential things to the limit of favourability, or by maintaining reactive inimical factors, such as the attack of natural enemies, at the limit of tolerance.

It would be difficult to picture more exactly the position mathematically described in the Lotka-Volterra equations.²

Populations, says Nicholson, regulate their densities by depleting essential things to the limit of favourability or by maintaining inimical factors at the limit of tolerance. If we ask how they do this the answer Nicholson gives is that they do it by "competition." The word "competition" said Dobzhansky (4) has an "emotional" significance and should be eliminated. Nicholson is able to give an etymological derivation that agrees with his usage, but the fact is that the current meaning no longer follows the etymology. The term "competition" inevitably brings to the mind the idea of a contest between human beings involving planning and deliberation. However, for Nicholson, competition is simply any condition in which the existence of individuals constitutes in some way a disadvantage to their fellows increasing the mortality rate in the population. It is, as Solomon has said, merely a statistical conception. Nevertheless, competition, in Nicholson's sense is another element of primary importance in his theory of populations. From his point of view I think it can hardly be over-stressed. There is one well-known remark made by Nicholson in which his fundamental views are epitomized and which enables us better perhaps than any other passage in his writings to understand the general line of his thought. This is the

² Nicholson's bibliographical references give a somewhat inaccurate idea of the historical development of this subject. The first mathematical representation of a parasite-host relationship, corresponding to the fundamental ideas of Nicholson was that produced in 1923 by Lotka (9) after a study of the equations for parasite host relations which I published in 1922 (17 to 20) and was suggested by those equations. Nicholson says that Verhulst, Pearl, Volterra, and he, each independently chose the same factor as being of primary importance in population regulation, this factor being the progressive decrease in the favourability of the environment which is associated with progressive increase in population density. We need not cast doubt on Nicholson's belief that he was an independent discoverer of this idea. However, it should be noted that the basic work of Volterra (27), dated in 1931 by Nicholson, was actually published in 1926. The basic work of Lotka as I have said appeared in 1923 and not in 1925, as Nicholson's reference would indicate. He quotes a work of Pearl published in 1926, but one of the basic papers of this author came out in 1920 (13). As for the work of Verhulst, it actually appeared in 1838 (26).

statement about percentages of mortality. In his 1933 paper Nicholson (11) claimed that

if an attempt be made to assess the relative importance of the various factors known to influence the population, no reliance whatever must be placed upon the proportion of animals destroyed by each. Instead, we must find which of the factors are influenced and how readily they are influenced and how readily they are influenced by changes in the density of the animals.

Food shortage is a mortality factor. The greater the density of the animals in a given environment the greater the shortage becomes and the more intensely it will act as a mortality factor. On the other hand the air temperature, though unfavourable, will not under ordinary circumstances be rendered less favourable by an increase in density.

Organisms, according to Nicholson, regulate their densities through the mechanism of competition. Competition, as we have seen, is in the view of Nicholson any condition in which the existence of individuals constitutes in some way a disadvantage to their fellows, increasing the mortality rate in the population.

We are therefore impelled to ask what is the reason for competition. Why does competition assume such vital importance with respect to populations? The answer of Nicholson here is that competition is of vital importance because animals search for the things they require at random. In this first paper Nicholson definitely asserted that though individual animals may follow a definite plan when searching, searching within the population is unorganized and therefore random. Perhaps he has now slightly modified his views, for he says that his conclusions are not completely dependent upon the assumption of random searching but that essentially the same conclusions which emerged from this postulate are derived from the well-established fact that growing populations inevitably and progressively deplete some requisites. Nevertheless he still says that random searching is a reasonable primary postulate when applied to entomophagous parasites. He says also that this is the same simplifying hypothesis which was used by Verhulst, Pearl, Lotka, and Volterra in their equations, and as we have seen, these equations do correspond to the facts of population change, as Nicholson envisages them.

Thus, to sum up, for Nicholson,

Populations are self-governing systems. They regulate their densities in relation to their own properties and those of their environment. This they do by depleting and impairing essential things to the threshold of favourability, or by maintaining reactive inimical factors, such as the attack of natural enemies, at the limit of tolerance.

The mechanism of density government is almost always interspecific competition. Governing reaction induced by density change holds populations in a state of balance. This balance is commonly a state of oscillation about the level of the equilibrium density, which is forever changing with

environmental conditions. Population densities can be governed only by factors which react to density change, but factors uninfluenced by density may affect it by influencing the level at which governing reaction adjusts population densities.

I have made an effort in the preceding parts of this paper to show how Nicholson's ideas have been developed, and I hope that the summary which I have just given in his own words will now be comprehensible.

The most persuasive argument he uses, is, I think, that if population densities are "regulated," then this can only be by factors the intensity of whose action increases with density increase and decreases with density decrease. Reactive factors having the properties just described, must therefore regulate population densities, and other factors can only determine the average levels around which this regulatory process occurs. Stated in this way this simple proposition sounds like an axiom although its substance is of course not at all new.

I do not think it is an axiom. When we consider Nicholson's summary of his views we see that the subject has become entangled in philosophical and semantic difficulties. To get a clear view of the problem it is best to return to reality. Let us take the case of 20 caterpillars feeding on an isolated perennial plant, capable of supporting only one caterpillar. The caterpillars eat the plant to the stalk, 19 die of starvation and one survives. Then, in the following year, if the plant has not been damaged too severely, it grows up again, and if the caterpillar has survived, transformed, and mated, the adult may lay 20 eggs which produce caterpillars which again "compete" for the inadequate food supply, and they again "regulate their densities" by reducing their "requisite" to "the limit of favourability."

This language is quite inappropriate to the discussion of the facts described. The population is referred to as if it were an entity, but as an entity it exists only in the mind, having merely the status of a concept. Nicholson has reified or hypostatized it and has thus created a quite unreal being to which he then ascribes the power of self-preservation or adaptation. By such misleading language the reader is led to regard self-regulation as a fundamental property of populations. In fact, however, the only real beings that exist in the real world are individual organisms, and they alone (if we except the animal societies) have the power of self-regulation or adaptation. In the population of caterpillars every individual simply does the best he can for himself, without paying any attention to the others. For this reason, even where "competition," in Nicholson's sense, exists, the results that follow from it are entirely a matter of chance. The 20 caterpillars all might and often do, die of starvation even without consuming all the food available. The geological deposits are packed with the debris of species without number that have thus "regulated" themselves to extinction. It can, of course, be said that if the 20 caterpillars do all die of starvation, they have in fact adjusted the density of their population in conformity with environmental conditions. Carried to this extreme, the

conception assumes a slightly comic aspect; nevertheless we have arrived at this point simply by following the principles to their logical conclusion.

Whether populations deplete the environment merely to the limit of favourability or beyond it is, as I have said, simply a matter of chance. Because individual organisms possess the power of self-regulation or adaptation and have sometimes a reproductive rate that allows for even an enormous mortality, and are fairly tolerant of certain variations in environmental conditions, the situation is somewhat weighted so to speak in favour of the survival of most species (though not of most individuals) at a given time.

Futhermore, we can define the general characteristics of a region, at least in a broad general way, which means that the conditions of life remain somewhat the same and certain organisms can properly be described as common or rare. The environment as a whole tends to allow them to exist at any time at a certain numerical level though how this will change is often impossible to predict. However, everyone with some field experience can recall instances in which the reckless pursuit of their individual interests by animals has led to their local extinction. In the infestation of the Bermuda Juniper by the imported scale *Carulaspis visci* the entire population of junipers on many small islands was completely exterminated by the scale which then died out with its parasites. I have already mentioned briefly elsewhere the case of a small sawfly population I once observed on a small isolated pine tree. The larvae in this colony were attacked by a tachinid which was probably *Euphorocera claripennis* Macq. The female parasites laid eggs repeatedly on the larvae so that these were eventually very heavily parasitized with the result that only one or two weak, very under-sized flies eventually emerged. These died without mating and all the sawfly larvae died. Such cases clearly show that we cannot depend on the insect hosts of insect parasites to maintain their natural enemies only up to the limit of tolerance.

I must again insist on the fact that by the use of this language, a phenomenon in which the population is merely a passive factor, is described as if it were exerting positive adaptive activity. Suppose we have 10 cows in a field. They eat the grass until it is all used up. The final result is that nine cows die and one survives. In the presence of this one cow the grass recovers sufficiently to maintain it in existence. Certainly the two species: the cow and the grass, have both survived; but the population, like the species, in itself, is merely a concept. It exists, of course, in the real world, as a collectivity, but it is unified only in the mind, and it is therefore only in the mind that it exists as an entity. To describe it as a self-regulating system, like an animal body, is merely playing with words. Neither the grass population nor the cow population is really responsible for the preservation of its own existence in any real sense. The eventual result is an accident attributable to a particular conjunction of independent causal series. If things had been very slightly different the cows might

have eaten the grass up so that all died. If we desire to find principles on which to base our theories of populations we must look for them, in the first place, in the behaviour of real entities, which means, in the behaviour of individual organisms. The fact that individual organisms seek to satisfy their needs without regard to the interest of their fellows, or of the population as a whole, does not always lead to catastrophic consequences. In fact situations of the kind I have just described are on the whole infrequent as I think most entomologists will agree. The fact that insecticidal treatment is frequently necessary to reduce insects damage to trees and crops is of no particular significance in this connection; because damage that is significant from the economic standpoint is usually far below what Nicholson calls the limit of favourability. Treatment is applied before the density of the species is very high and indeed is often applied before it is definitely known what this density would be without a treatment. When we consider the actual position as we see it in nature we have to agree with the many authors who have pointed out that there is little indication that insect increase is limited by the supply of food. In the case of the leaf-feeding Lepidoptera, for example, we generally find only a few specimens of any particular species in the middle of a food supply sufficient to support vast numbers.

Nicholson has himself called attention to this difficulty. He says (12, p. 49),

almost of all kinds of plants are attacked by one or more species of animals tend to reduce their food supplies to the threshold density at which they can barely find enough food to maintain themselves and to produce sufficient offspring to replace them at sparse distribution of their food plants whereas observation indicates that terrestrial plants succeed in occupying fully, practically every site in which conditions are suitable for plant growth. The question is, how can these apparently contradictory conclusions be reconciled?

He goes on to say that,

Sometimes herbivorous animals do limit their densities by reducing their host plants to such scarcity that they have great difficulty in finding them as happened as a result of the introduction of *Cactoblastis cactorum* into Australia to attack *Opuntia* spp. and of the introduction of *Liothrips urichi* into Fiji to attack *Clidemia hirta* [Simmonds (15)]. It is possible that such fragmented distribution of plants caused by the action of their enemies (notably insects) is far commoner than is realized, for the influence of the enemies is inconspicuous when balance exists and both insects and their host plants are scarce and widely scattered. However, it is quite evident that this situation is not general and that usually animals are prevented by other factors from directly limiting their food supplies. One mechanism is a destruction of weakened herbivores by natural enemies, in the way, already referred to, which prevents the eating out of pastures and causes the herbivores to maintain populations near the maximum density the pastures could possibly support. Insect enemies of plants on the other hand, appear generally to be reduced by their own enemies to the threshold level for these, which commonly means that their effect upon plants is not very signifi-

cant. Probably many factors other than the availability of their food plants and the attack of natural enemies limit the population of some plant feeding animals but there seems very little doubt that the major influence which prevents animals reducing vegetation on the earth to extreme sparseness is the attack of phytophagous animals by enemies of their own.

These remarks, if I interpret them correctly, constitute an acknowledgment that the insect enemies of plants do not usually appear to be depleting and impairing essential things to the threshold of favourability, for the plant food is assuredly a "requisite" for the phytophagous insect. However Nicholson endeavours to bring the observed facts into agreement with his theory by suggesting that the insect enemies of plants are generally reduced by their parasites and predators to the threshold level for these, which means, to a level below which the extinction of both the phytophagous insect and its insect enemy would occur.

If it is true that populations regulate their densities by depleting and impairing essential things to the threshold of favourability then we should expect to see phytophagous insects, unless restrained by other factors, making constant and very obvious and severe inroads on their food supplies, because, if they really deplete their food to the limit of favourability a very slight additional consumption of food would lead to their own extinction. This principle will hold also with parasites and predators because according to Nicholson these are maintained by the populations at the limit of tolerance.

However in my experience, even when working with the Lepidoptera, which is without doubt the group in which parasitic attack is most frequent, one seldom finds a high proportion of the population infested by parasites. Just as one has to hunt quite extensively through a woodland to make a satisfactory collection of caterpillars of a given species so one has to hunt through a large number of individual caterpillars in order to obtain the various stages of an insect parasite so as to work out its life history.

Nicholson says, it is true, that

if an attempt be made to assess the relative importance of the various factors known to influence the population, no reliance whatever must be placed upon the proportion of animals destroyed by each. Instead we must find which of the factors are influenced and how readily they are influenced by changes in the density of the animals.

But what this means is, that the factual evidence we can obtain at any particular moment in nature, has not necessarily any significance in regard to the problem we are trying to solve. To discover the inner meaning of the facts, a very lengthy and arduous investigation has to be made. In cases where this investigation has not been made, we simply have to rely on faith: "the substance of things hoped for, the evidence of things unseen," as St. Paul says. When we find half a dozen caterpillars on a tree that can support ten thousand, our impression certainly is not that the caterpillars

have utilized the available requisite to the limit of favourability or that there is anything we could reasonably describe as competition.

The answer to this difficulty might be in the first place that there is occult competition: for example, for oviposition sites, and that the low ratio of larvae to food supply is a result of this "competition." Another reply might be, that if there is no competition for the moment, it will occur in the course of future generations. Nicholson would I think agree since he says that if "accumulation of a requisite in the absence of a given species exceeds the threshold density, the introduction of this species will be followed by population growth, for the animals at first obtained more than their maintenance quota." We might feel inclined to recall also that according to Nicholson populations are always in a state of balance. He said in his 1933 paper that they must "exist in a state of balance, for they are otherwise inexplicable." The very fact that the population has a determinate value (is composed, for example, of 10 caterpillars) proves that it is in a state of balance. The mechanism producing balance is almost always intraspecific competition. Therefore, if populations are always in a state of balance, the population of 10 caterpillars is in a state of balance and if the mechanism of that balance is intraspecific competition, then competition must be responsible for the balanced condition of this population.

Let us consider the state of this population at 3 p.m. on July 1, 1955. According to Nicholson it is then in a state of balance because populations are always in a state of balance. If this means that the population is as it is, because things are what they are, well and good. However, Nicholson (12) says that "the characteristic of balance is sustained and effective compensatory reaction." He says also that "Balance is commonly a state of oscillation." However considering the population of 10 caterpillars at the instant designated and admitting that it is in a state of balance, we must nevertheless admit that we cannot see in it any sustained or compensatory reaction nor can we discern in it a state of oscillation. The most extreme concession we can make would be that intraspecific competition has in some way produced a condition we now see, but this would be merely a deduction from the theory, not an expression of the actual facts known to us.

We have observed that there are 10 caterpillars on the tree, although there is apparently enough food for a thousand caterpillars. We can assume, in deference to the theory, that though there are only 10 caterpillars this is because there was an intense competition for oviposition sites as a result of which only 10 eggs could be deposited. However, if we consider the matter objectively we have to admit that this may have been attributable to a gust of wind which carried off the ovipositing moth and blew it into an adjacent pond or to a downpour of rain which washed it into the ground or to some other accident which terminated its existence before it finished laying its eggs.

Suppose now before the end of the life-cycle another downpour of rain

destroys 8 of the 10 caterpillars leaving one to transform into a female moth and the other to transform into a male moth. The population will then have gone through a cycle at the end of which it is reduced to the state in which it was in the beginning. We can imagine a case in which the existence of the 10 caterpillars has constituted competition, from which, again, only two caterpillars survived to transform. The end result of the two phenomena would be the same, but we should have to say that in one case the population is "controlled" and in the other case it is not it has merely "varied."

Perhaps it may appear that I am merely trying to make what are usually called debating points. I have suggested that Nicholson's views on balance, as he has stated them, for example, in the summary preceding his 1954 paper, correspond to the mathematical model of the Lotka-Volterra equations. It must be recognized that Nicholson himself criticises this model, making the very reasonable objection that the instantaneous reaction it implies does not exist in nature except perhaps in organisms like bacteria and protozoa, at least this seems to me to be the meaning of his objection. Since this is his view, my objections may seem beside the point. However, the criticism I am really making is, that in spite of his objections to the model on factual grounds, he nevertheless seems to return to it in formulating his general conclusions and his views on balance, which become quite intelligible if interpreted in terms of the model but otherwise are inexplicable.

I have suggested that under certain circumstances climatic changes have a considerable influence on populations. Nicholson, if I understand him correctly, holds that climate does not and cannot govern population densities. By this he means, I think, that it cannot "regulate" population densities. There has, of course, been a rather general agreement amongst entomologists that the destructive effect of climatic factors is more or less independent of the numerical value of the population on which they act so that a heavy rainfall for example will destroy the same proportion of the caterpillars on a tree whether there are 100 or 1000.

Nicholson takes a case in which all other factors on the environment are also nonreactive, that is to say, their destructive effect does not increase in intensity as the population increases in density and decrease in intensity as the population decreases in density. In such a case, says Nicholson, if all conditions are favourable to the organisms, their populations will grow without check and so in any long period of time will increase to indefinitely large numbers. This, he says, is true even though the permitted rate of geometrical increase is very low and irrespective of whether the environment remains constant or fluctuates violently about this average favourable level. This argument appears to be sound provided the oscillations above and below the average level are uniform. However, occasional unduly severe conditions or a succession of moderately severe conditions in the same year might easily bring the population to the level at which it was at the beginning of the period considered. Nicholson goes on to say that, conversely, if conditions

are unfavourable, however slightly, the population must tend toward extinction in long period of time. Even if the degree of favourability were maintained at the minimum, so just permitting the replacement of animals as they die, the absolute density would be independent of the environment, being simply the initial density in a constant environment or fluctuating about this density in a fluctuating environment. In fact in all three situations the absolute density at any moment would be merely a function of the initial density and of the subsequent history of increase and decrease, in relation to the varying favourability of the environment. A change in density would be related to change in favourability; but absolute density would be independent of the conditions prevailing at any moment. This, if I understand Nicholson's idea, is because the unfavourable climatic conditions would destroy a certain percentage of the population, no matter what its density. Thus, if we began with 1000 individuals and they increased to 4000, an unfavourable climatic change at this moment might kill off 3000 leaving only 1000, but if it had occurred at the beginning of the period it would have killed off 750 leaving only 300. The absolute density at any moment would then be independent of the conditions prevailing at that moment in the sense that the population on which the climatic factor had to act would depend on its density to start with and the increase that happened to occur in the interim.

Nicholson is here following out his principle that "if an attempt be made to assess the relative importance of the various factors known to influence the population, no reliance whatever must be placed upon the proportion of animals destroyed by each. Instead we must find which of the factors are influenced and how readily they are influenced by changes in the density of the animals." According to this principle the importance of a mortality factor is determined by the degree of its density dependence. However the existence of such factors was not overlooked by previous workers, and Nicholson's view about them stands out more especially because of its intransigent character. In the ordinary operations of the economic entomologist the principle is not and cannot usually be applied. For example if I spray my clothes-closet with an insecticide to kill clothes-moths, a 99 per cent kill will be satisfactory to me, even though I thus reduce the competition among clothes-moths and relax intraspecific pressure, thus giving the clothes-moth population an opportunity to build up again. If a fruit grower sprays his orchard for codling moth and saves 95 per cent of the crop, thus making a substantial addition to his income, the percentage mortality he has obtained will, to him, be the important index of the controlling factor of the spray he has used. If low temperature or heavy rain wipes out an injurious insect at an early stage in its development, reducing economic damage to a minimum, the agriculturist who has benefited from the action of this factor will not care whether it is "reactive" or not. He will consider that the percentage mortality has been the real index of its importance and in this view he will be perfectly correct. Economic entomologists and agriculturists in

general work season by season or, if you like, generation by generation, and it is only with respect to a fairly extended period of time or a series of generations that the principle laid down by Nicholson applies. The ecologist who is studying the variation in population and the causes of mortality that operate throughout the life-cycle is obliged to adopt the same viewpoint. If he finds that in a certain generation of insects, unfavourable weather has wiped out a large part of the population, he is obliged to recognize that weather has been one of the important factors determining population density. If he is then told by Nicholson and his adherents that nevertheless populations do not control density and that, to control density, density-dependent factors are required, he can only reply that at all events density independent factors really determine density even if they do not "control" it in the sense that they "regulate" it. We are in fact struggling here again, with pseudo-problems, arising partly from semantic difficulties. Nicholson does not deny that density independent factors have a determining influence on density. Climate, he says, has a profound effect on density. Climate also limits the distribution of species. It seems clear that if by climatic influences a species is excluded from certain areas we can say that in these areas the density of the species is reduced to zero. The word reduction is not improper in this connection because as a result of the fact that climatic conditions often change very drastically a species might occupy a certain habitat for a time and then be exterminated, as would individuals who happen for various reasons to wander into the habitat or are carried into it.

In my 1939 paper (23) I said that the discontinuity and variability in habitats produced by the physical factors is undoubtedly the primary extrinsic factor of natural control. It will now be amply clear that the argument in which we are engaged turns to an important extent on the meaning of the word "control." By control Nicholson means regulation or government, whereas control in my view simply refers to the fact that no organism increases without limit. To this Nicholson has replied (12) that the discontinuity and variability of habitats does not inconvenience organisms to any extent; "most species," he says "are well adapted to cope with such fragmentation." These adaptations, in his view, enable animals to occupy fully all favourable sites, and in these sites the populations are governed by density reactions.

But if that is so, how is it that a rarefaction of the host population, according to the mathematical formula adopted by Nicholson, determines a fall in the reproductive rate, while an increase in host density causes it to rise? The rarefaction in the host population with respect to a parasite is simply a stage in or, rather, an example of, the progressive fragmentation of the habitat. To an onlooker or to a disinterested observer it may now appear that whereas, at one point, I stress, in opposition to Nicholson, the ability of organisms to find suitable habitats, in order to refute the thesis that animals search for the things they require at random, now Nicholson is arguing in favour of the adaptive abilities of animals in the hope of demonstrating that

climatic factors do not control populations! On the other hand, by insisting that the fragmentation of habitats produced by physical factors is the primary extrinsic factor of natural control I appear to be denying to animals the adaptive ability which I had at first attributed to them!

This rather paradoxical situation arises I think in the first place from the fact that the views of Nicholson and myself are in many ways similar, because we are both anxious to take all the available facts into account, and yet they differ in certain subtle but extremely important respects. The difference is, I think, not just what might have been expected from the general trend of the arguments on both sides. It is, that when all is said and done the factor of chance plays a much greater part in the world as I see it, than it does as in the world as Nicholson sees it. It is true that I have frequently stressed the adaptive abilities of animals more than has Nicholson. I feel indeed that the adaptive abilities of animals are extremely remarkable. Nevertheless, I am convinced that in spite of these abilities many of them find it extremely difficult to survive because their possibilities of existence are quite restricted by the specificity of their requirements and by the great diversity and fragmentation of their habitats, both in space and time. They have to move through a network of random events.

Random events are attributable to what we call chance. Chance consists essentially in the intersection of independent causal series. For example, while one man is walking to the public library at 10 p.m. July 3 he meets a friend who is walking to the theatre. This meeting was not intended by either man. Nevertheless, some benefit may result to one or both of the men from the meeting. This benefit was not intended in the original intention of either of the men. The meeting was an accident; the fact that it produced results beneficial to one or both of the men is an additional accident. The probability of the meeting was small, of the benefit still smaller. The more precisely defined the benefit, the less the chance that the meeting would produce it. It might be expected to produce the pleasure arising in a general way from the meeting of friends, but it was very much less likely that one friend would give the other the exact component required for the repair of his broken-down radio. If this very surprising event happened at 10 p.m. on July 3 we should certainly not expect it to happen at 6 p.m. on July 5. Furthermore, the man on his way to the library might meet 20 other men without deriving any benefit, either general or special, from these encounters.

The Darwinian geneticists claim that every single specific characteristic of an organism is positively adaptive. The organism is therefore fitted to the environment, as Fisher (7) has said, like a complicated key to the lock. If this is so, we must certainly realize, if we consider objectively the fact that the environment is at no two points and at no two moments exactly the same, that the individual organism that survives to reproduce is extremely fortunate. The larva which has been lucky enough to develop in a completely suitable environment may find, at the time of pupation, that a radical change has occurred, not necessarily through its own activities but attributa-

ble either to the activities of other organisms or as a result of qualitative and quantitative change in the physical factors. Such changes are continuous and of tremendous complexity. Their existence, which is one of the most obvious facts of nature, makes it quite superfluous to postulate for the control of organisms such so-called reactive factors as natural enemies and the depletion of the food supply. I do not, of course, deny that in certain rather infrequent cases, depending on a particularly favourable conjunction of the movement of the organism and favourable physical factors, multiplication may proceed to a point where the exhaustion of the food supply or the assemblage or multiplication of natural enemies annuls the advantage the species has gained. But so far as the objective evidence goes, it gives no solid grounds for believing that populations normally and constantly regulate their densities by depleting requisites to the limit of favourability or by maintaining the attack of natural enemies to the limit of tolerance.

To test the soundness of his views Nicholson has carried out experimental studies. In his experiments he has obtained population changes of the general type that might be expected from his theoretical principles. It must be noted, however, that he relies chiefly on experiments with blow-flies such as *Lucilia*. The most important facts described by Nicholson were published in 1932 by Salt in his paper (14) on the natural control of *Lucilia sericata* Mg. The whole subject was treated in very much greater detail in a large paper by Ulyett (24). Salt concluded from his experiments that *Lucilia sericata* is one species that normally, in England and France, completely saturates its environment, the factor that chiefly limits the increase of its population being the shortage of carcasses. He added that the potential reproductive rate of *Lucilia* is so high that even if more carcasses became consistently available they would soon be over-blown and the same competition and elimination would occur.

However this example merely illustrates the fact that, as has always been recognized, the so-called reactive factors are operative at certain times and places with certain species. The behaviour of the blow-flies is clearly unusual. The facts we observe with this species cannot be generalized and then taken as the foundation of a comprehensive theory of natural control, I mean, as an explanation of the fact that no organism increases without limit.

Some ecologists have claimed to find in data they have collected with respect to field populations evidence of fluctuations which might be explained on the basis of theories like that of Nicholson. However after a careful study of the material Cole (3) has come to the conclusion that the preferred interpretation of population cycles should be that they are essentially random fluctuations with serial correlations between the population of successive years. "If one wishes to postulate mysterious causes for population cycles," says Cole, "there is no way to disprove their existence; the most the writer could say of such hypotheses is that at the present time he considers them unnecessary."

My general conclusion, therefore, is that populations are not self-govern-

ing systems which typically regulate their own densities and maintain themselves in existence by depleting requisites only to the limit of favourability and maintaining inimical factors only to the limit of tolerance. They are collections of individuals each of whom feeds, grows, and multiplies without regard to the interests of his fellows or of the collectivity. If an organism does not multiply without limit this is because it is restricted by its intrinsic specific limitations in a world which is made up of an ever changing complex of other specific and limited entities. Climatic and edaphic factors are the basis of the environmental diversity, producing not only the fragmentation of habitats but a constant change in their character and location. This is the primary extrinsic factor of natural control in the sense in which I have used this expression. In extreme cases, where a chance conjunction of favourable circumstances has led to long continued increase, the induced shortage of requisites or the multiplication of natural enemies drawn in by the mass attraction of the host population, may reduce this population; but such cases are clearly exceptional. Populations, therefore, are not truly regulated but merely vary, although indefinite increase is unlikely and in the long run will become impossible. The variation sometimes assumes a cyclical aspect, but this is usually attributable to chance, not to factors producing oscillations according to definite laws. The usual variation in the numerical value of field populations from year to year corresponds to curves based on numbers selected at random.

It is possible to formulate some incontrovertible principles about the relation between organisms and their environments; but we cannot deductively obtain from these principles a detailed prevision of natural events. What they actually indicate is that because of the contingency in nature, we are forced back to the method of observation and experiment, and it is on observation and experiment that we must rely to reach a realistic understanding of the changes in populations. It is on this solid groundwork that our biological control work must be based.

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EFFECT OF PESTICIDES ON BALANCE OF ARTHROPOD POPULATIONS¹

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INTRODUCTION

This review summarizes information on the effect of pesticides on the balance of arthropod populations with special reference to phytophagous pests. While no special review on this subject has appeared before, articles by Greenslade (80), Massee (137, 138), Pickett (161), Satchell (178), Schneider (210), and Solomon (185) as well as the book by Brown (24) have in recent years brought together a good deal of the relevant literature. No attempt will therefore be made to catalogue the older literature, but it is planned to evaluate critically the more recent investigations.

In the majority of cases when pesticides are sprayed on crops to control phytophagous insect outbreaks, a reduction of population of the pest is obtained without alarming repercussions by the arthropod population. But the natural environment is an unstable system. The number of insects and mite species present varies, and the interrelationship amongst the constituent species is complex. Since the interdependence of the various arthropod species comes to an equilibrium, a "balance," a removal of one or, more frequently, of several species by the application of pesticides can have profound influence on the other arthropods present.

Such disturbances of the balance of arthropod populations can be put into two groups: "short-term" effects which take place during one vegetation period, of which the most notable is the resurgence of pest population soon after the chemical treatment; and the more insidious "long-term" effects which become obvious only after a number of years, sometimes culminating in the appearance of strains of arthropods resistant to the pesticide.

These phenomena constitute a major weakness in the chemical control of pests which has raised the question whether it is not an overrated method which is indeed rapidly stultifying itself. As chemical control is for all but a minority of pest outbreaks the only immediately practical control method available, this weakness must be urgently remedied.

To this end, ecological studies, research on alterations of the application method in accordance with results of the ecological studies, and modification of the toxicological approach, have been embarked upon. Real progress is now being made in this field, but there are as yet insufficient critical investi-

¹ The survey of the literature pertaining to this review was completed in July, 1955.

² It is a pleasant duty to acknowledge the help which many workers in this field have given the reviewer and in particular to Drs. A. M. Massee and P. DeBach who have read and criticized the manuscript.

gations on several of the more important phenomena, so that their generality cannot always be assessed.

When this review concentrates on undesirable sequels of chemical treatment, they will be kept in the right perspective if we do not lose sight of the many successful and uneventful chemical control operations which are being executed annually on a vast acreage.

RESURGENCES OF PEST POPULATIONS AFTER SPRAY TREATMENT

In spite of a good initial kill at the time of treatment, chlorinated hydrocarbons, parathion, para-oxon, sulphur, lime sulphur, copper carbonate, calcium arsenate, derris, zinc sulphate, and thiuram, even carrier materials and diluents used on orchard or field crops, at times bring about a tremendous increase of the pest against which they have been applied, or of other phytophagous species present only in small numbers at the time of treatment. These increases are called resurgences of arthropod population or "flare backs." Resurgences (summarized in Table I) have been recorded in temperate, sub-tropical, and tropical climates for over 50 species of phytophagous arthropods belonging to the families of Tetranychidae, Eriophyidae, Tarsonemidae, Coccidae, Aphidae, Aleyrodidae, Cicadellidae, Noctuidae, Tortricidae, Olethreutidae, Trypetidae, Agromyzidae, and Collembola.

There are about 5,000 insect and mite species of economic importance; of these, about 50 species have so far shown resurgences, that is, roughly 1 per cent. The spread of this phenomenon over a number of very different groups of phytophagous arthropods and its occurrence after the application of several types of pesticides with different modes of action and in a wide variety of climates, indicates that chemical control in these cases upsets the population dynamics involved. To explain the phenomenon, three hypotheses have been put forward: (a) The reduction of natural enemies by the pesticide, along with the pest. (b) Favourable influences of pesticides on phytophagous arthropods. (c) The removal of competitive species.

THE REDUCTION OF NATURAL ENEMIES (SHORT-TERM EFFECTS)

When dealing with the short-term effect resulting in a reduction of natural enemies, it is convenient to differentiate between resurgences of pests against which a pesticide treatment had been applied, and resurgences of other phytophagous arthropods which were triggered off through such treatment but which before the treatment were present in unimportant numbers.

Unfavourable influences on natural enemies of the pest against which the control was aimed and their resurgence after spraying.—Many insecticides and fungicides now in general use have a destructive effect on the natural enemies of the phytophagous species and often eliminate the predators and parasites; the extensive literature is tabulated in Tables II and III. The available information refers chiefly to adults in the case of parasites; less is known about the influence of insecticides on the larvae of parasites. Normal emer-

gence of adult parasites after treatment of pests containing internal parasites occurs after HCN fumigation (69), short exposure to nicotine vapour (168), spray treatment with miscible oil, nicotine sulphate, and lime sulphur (154).

There are differences in the relative toxicity of insecticides between the host and internal parasite. Holloway, Henderson & McBurnie (102) found that fumigation with HCN resulted in lower relative mortality of pupal *Comperiella* sp. than of its host, the yellow scale, whereas oil sprayed caused nearly the same mortality of parasite and scale. Similar results were obtained with *Metaphycus helvolus* (Compere) and its host, the black scale. The oriental fruit fly, *Dacus dorsalis* (Hendel), was found more susceptible than its internal parasite, *Opius oophilus* (Fullaway), in topical application of dieldrin, equally susceptible to isodrin, and more susceptible to aldrin, endrin, chlordane, and lindane. In the soil treatments the parasite and fruit fly larvae were equally susceptible to aldrin, dieldrin, endrin, chlordane, and lindane [Tamshiro & Sherman (195)]. In topical and soil treatments both parasite and fruit fly larvae were highly resistant to DDT.

While there is ample evidence of destructive effects of pesticides on the natural enemies, the criteria required to justify the conclusion that the removal of parasites and predators is responsible for the resurgence of the pest population are: (a) a negative correlation between the number of natural enemies and pests, (b) proof that the natural enemies were the limiting factor to the density of the population, and (c) an exclusion of stimulating influences of the pesticide on the pest directly or via the plant.

There are many records of negative correlation between the population density of pests and their natural enemies. Some of the best examples are a result of the research work by DeBach (45 to 54), Bartlett (8 to 14), Collyer (35 to 39), Huffaker (108 to 111), and Wallace (200). DeBach (45) even used DDT and other pesticides with preferential toxicity to natural enemies over the hosts as one of the methods to determine the relative efficiency of biotic factors limiting the population density of the pest. He also obtained experimental proof that DDT residues cause no direct or indirect stimulation of either the California red scale, *Aonidiella aurantii* (Maskell), or the yellow scale, *Aonidiella citrina* (Coquillett), which results in population increase (54).

The possibility of DDT residues or a physiological effect of DDT on the tree or both making the environment more favourable to the citrus red mite, *Metatetranychus citri* (McGregor), was considered by DeBach, Fleschner & Dietrick (53). They deduced from their field data that there was little or no effect of DDT other than that resulting from the decimation of natural enemies. As far as the fruit tree red spider, *Metatetranychus ulmi* (Koch), is concerned, Hueck and co-workers (107) and Hueck (106) came to different conclusions. In the light of the discovery by Hueck *et al.* (107), Fleschner (66), and Kuenen (125) some of the results of the investigators mentioned above might be subject to the criticism that neither favourable influences by the insecticide on the biotic potential of the phytophagous arthropod, i.e.,

TABLE I

RESURGENCES OF ARTHROPOD POPULATIONS AFTER THE APPLICATION OF PESTICIDES

Phytophagous Arthropods	Aldrin	BHC	Calcium Arsenate	Chlordane	Copper Carbonate	Cryolite	DDD	DDT	Deris	Dieldrin	Hepachlor	Lime Sulphur	Para-oxon	Parathion	Petroleum Oils	Phenothiazine	Sulphur	Tar Oils	Thiram (T.M.T.D)	Toxaphene	Zinc Sulphate	Authors
Tetranychidae																						
<i>Tetranychus telarius</i> (Linnaeus)	+	+	+				+	+		+										+		7, 51, 121, 122, 129, 188
<i>T. mcDanielis</i> McGregor								+		+												7
<i>T. schoenlet</i> McGregor								+		+												103
<i>T. pacificus</i> McGregor								+		+												7, 144, 145, 159
<i>Metatetranychus citri</i> (McGregor)	+		+			+		+		+											+	47, 51, 58, 59, 115
M. ulmi (Koch)								+														7, 18, 35, 76, 134, 138, 139, 161, 164
<i>Oligonychus ununguis</i> (Jacobi)								+														100, 141
<i>O. yohkersi</i> (McGregor)								+														166
<i>Bryobia praetiosa</i> Koch								+														7, 82, 101
<i>Eurytetranychus buxi</i> (Garman)								+														141
Eriophyidae																						
<i>Vasates lycopersicae</i> Massee								+														93
<i>Phytocetrus olivora</i> (Ashmead)								+														87, 93
Tarsonemidae																						
<i>Steneotarsonemus pallidus</i> (Banks)														+								111
Collembola																						
<i>Sminthurus viridis</i> (Linnaeus)								+														200
Coccidae																						
<i>Coccus hesperidum</i> Linnaeus								+		+												13, 51, 62
<i>Icerya purchasi</i> Maskell								+		+												46, 51
<i>Planococcus citri</i> (Risso)						+		+														20, 51, 87
<i>Pseudococcus adonidum</i> (Linnaeus)			+	+				+														45, 51
<i>P. maritimus</i> (Ehrhorn)								+														55
Chrysomphalus ficus Ashm. (<i>comidum</i> auct.)								+														87, 158
<i>Eulecanium corni</i> (Bouché)			+					+				+										4
<i>E. prunosum</i> (Coccille)								+														147, 148
<i>Aonidiella aurantii</i> (Maskell)								+														20
<i>A. citrina</i> (Coccille)								+														51, 212

A. orientalis (Newstead)
S. citrinus (Coccille)

+

155
 20, 161

[illegible]

• Lead arsenate † Zinc arsenate ‡ Also methoxychlor.

TABLE II
INFLUENCES OF PESTICIDES ON PREDACIOUS ARTHROPODS

Predacious Arthropods	Aldrin	BHC	Chlordane	Copper Bordeaux	Copper Carbonate	Cyolite	DDT	Demeton	Derris	Diazinon	Dichloromaphthoquinone	Diethrin	EPN	Ferbam	Glyodin	Lead Arsenate	Lime Sulphur	Nicotine Sulphate	Nicotine (Fixed)	Para-oxon	Parathion	Petroleum Oils	Phenyl Mercuric Acetate	Schradan	Sulphur	Toxaphene	Zinc Sulphate	Authors
Thysanoptera <i>Lepidobrips mali</i> (Fitch) <i>Scolobrips sesmucialis</i> (Pergande) <i>Haplobrips foveae</i> Hood					o		+++				o				o	o	++	++	+		++	++	o		++		30 30 136	
							+++									+	+	+	+	+	+	+	o		++		136	
							+++				o				o	o	+	+	+	+	+	+	o		o		136, 186	
							+++				o				o	o	+	+	+	+	+	+	+	+	+		136	
Miridae <i>Hyaliodes harti</i> Knight <i>Diaphnidia pellicida</i> Uhler <i>Derocoris fascioides</i> Knight <i>Plagiognathus obscurus</i> Uhler <i>Cricoris saliens</i> (Reuter)						o	+++				o			o	o	o		+	+		++	++			o		136 136 136 136	
							+++				o				o	o	+	+	+	+	+	+	+	+	+		136	
							+++				o				o	o	+	+	+	+	+	+	+	+	+		136	
							+++				o				o	o	+	+	+	+	+	+	+	+	+		136	
<i>Phlophorus perplexus</i> Douglas & Scott <i>Campylomma verbasci</i> (Meyer) <i>Cyrtolihnius mundulus</i> (Breddin)		+			o	o	+++			o				o		o	+	+	+		++	++	o		++		136 136 44, 198	
							+++																					
							+++																					
							+++																					
Anthicoridae <i>Anticoris musculus</i> (Say) <i>A. nemorum</i> (Linnaeus) <i>Otus</i> sp. <i>O. tristicolor</i> (White)			+				+++							o		o	+	+	o			o			+		136 37 97, 152 89	
							+++																					
							+++																					
							+++																					
Nabidae <i>Nabis</i> sp. <i>N. fesus</i> (Linnaeus)		++	++				++																				97 27	
	++	++	++				++																		++			

TABLE III
INFLUENCES OF PESTICIDES ON PARASITIC HYMENOPTERA

Parasitic Hymenoptera	Aldrin	BHC	Chlordane	Copper bordeaux	Copper Carbonate	Cryolite	DDT	Demeton	Derris	Diazinon	Dichloromaph- thoquinone	Dieldrin	EPN	Ferbam	Glyodin	Lead Arsenate	Lime Sulphur	Nicotine Sulphate	Nicotine (Flux)	Para-oxon	Parathion	Petroleum Oils	Phenyl Mercuric Acetate	Schradan	Sulphur	Toxaphene	Authors
<i>Agallitis laticinctus</i> (Cresson)				○			++							○	○	++									○	++	136
<i>Allotropa</i> sp.							++							○	○	++									++	++	42, 104
<i>Anagrus</i> spp.							++																		++	++	3, 116
<i>Anagrus</i> <i>sydneyensis</i> Timberlake							++																		++	++	51
<i>Aphelinus mali</i> (Haldeman)							++																		○	++	202
<i>Aphidius</i> <i>typhlocyae</i> Muesebeck							++																		++	++	189
<i>Aphytis</i> <i>mytilaspidis</i> (LeBaron)							++																		++	++	136
<i>Ascogaster quadridentata</i> Wesmael							++																		++	++	136
<i>Clausenia purpurea</i> Ishii							++																		++	++	42, 104
<i>Coccophagus lycimnia</i> (Walker)							++																		++	++	73
<i>C. scutellaris</i> (Dalman)							++																		++	++	73
<i>Dacnusa gracilis</i> (Nees)							++																		++	++	213
<i>Dacnusa</i> <i>repae</i> (M'Intosh)							++																		++	++	171, 172
<i>Encarsia formosa</i> Gahan							++																		++	++	208
<i>Ephialtes</i> sp.							++																		++	++	136
<i>Encomys swederi</i> (Dalman)							++																		++	++	73
<i>Euderus</i> sp.							++																		++	++	136
<i>Macrocercus ancylionus</i> Rohwer							++																		++	++	182
<i>Metaphycus heliois</i> (Compere)							++																		++	++	64, 65, 212
<i>M. lounsburyi</i> (Howard)							++																		++	++	64
<i>M. luteolus</i> (Timberlake)							++																		++	++	11, 13
<i>Opis</i> sp.							++																		++	++	187
<i>Pachyneura</i> sp.							++																		++	++	187
<i>Prospaltella aurantii</i> (Howard)							++																		++	++	86
<i>Pseudophycus</i> sp.							++																		++	++	86
<i>Pseudomolodopa prima</i> Girault							++																		++	++	42, 104
<i>Tetraneura pretiosus</i> Timberlake							++																		++	++	86
<i>Trichogramma minutum</i> Riley							++																		++	++	51, 127
							++																		++	++	6, 23, 88, 112, 127, 136, 147, 179

KEY—○ = No influence; ++ = Mortality of 50%–95%; + = Mortality of 50%–95%; ++ = Complete elimination.

stimulation of reproduction, nor a decrease in the host resistance of the plant as described by Fleschner (66) were always sufficiently explored and excluded.

Studies on the effect of DDT and talc on the natural enemies of the long-tailed mealybug in California have shown these materials to be complex in their influence on populations of the arthropods present in the ecosystem. DeBach & Bartlett (51) found that light dosages of DDT did not affect the principal primary parasite of the long-tailed mealybug to an important extent. On the contrary, such light dosages eliminated its principal hyperparasite but also killed the predators of the long-tailed mealybug, the latter constituting the most effective biological-control factor. Lower dosages of DDT thus gave this mealybug selective advantages over the natural enemies which were not demonstrated at higher dosages where sufficient mealybugs were killed to offset the advantages observed with lower DDT application. In Ontario the introduced parasite, *Macrocentrus ancylovorus* Rohwer, appears to have been favoured by DDT and parathion applied for the control of the oriental fruit moth, *Grapholitha molesta* (Busck), in commercial peach orchards, while the native parasite *Glypta rufiscutellaris* Cresson has rapidly decreased in abundance during the period when DDT and parathion have been in general use (22).

The effect of DDT and BHC on the balance of the population of the alfalfa springtail, *Smynturus viridis* (Linnaeus), and its predator, the bdellid mite, *Biscirus australicus* (Womersley), were studied by Wallace (200) in Western Australia. Dressings of superphosphate alone or with DDT or BHC were applied and the arthropod populations studied during the season. On the plots treated with superphosphate only the springtail population declined steadily during the season and that of the predacious *Biscirus* was trebled. Similar population trends were observed on the BHC plot, though the initial decline of the springtail was rather more rapid. In the DDT-superphosphate plots, the number of springtails fell during the first 12 days, though not more rapidly than on the controls and subsequently showed no significant change. The bdellid mites were rapidly reduced and eventually died out completely, so that at the end of the season, *S. viridis* was five times as numerous as in the controls. In a second test the population of the springtail ended up on the DDT-superphosphate plot 19 times as great as in the "superphosphate only" plot; the bdellid mites were significantly reduced after 23 and 45 days, but there was no significant difference after 69 days. A negative correlation between the population density of the springtail and its predator was thus established, but an assessment of the effectiveness of the bdellid mite as a perimeter of springtail population and stimulating influences of DDT on the springtail natality was not attempted.

The consequences of deleterious effects of certain pesticides on the natural enemies were also demonstrated in a comparison of the effects on the balance of aphid populations by the selective systemic insecticide, schradan, with those produced by unselective organophosphorus insecticides, parathion, HETP, and para-oxon, which killed the natural enemies

of the pest as well as the aphids (171). Treatments with unselective insecticides were followed by resurgences of the aphids within 10 to 14 days which required retreatment. Where para-oxon was used commercially it produced on the sprayed fields the most enormous cabbage aphid outbreak within a fortnight after spraying which has ever been seen in England, although the mortality of the aphids at the time of treatment was very high. By contrast, the selective insecticide, schradan, which did not affect the natural enemies either directly or by ingestion of schradan-killed aphids, kept the cabbage aphid population at a low level without economic significance for the rest of the season (171).

While not conclusive, these data support the predator-parasite explanation for the resurgence of aphid outbreaks after parathion and para-oxon treatment, but convincing proof was obtained that the destruction of the natural enemies caused a resurgence by comparing changes in the pest population before and after removal of the natural enemies chemically and by hand. This method was followed by Huffaker & Kennett (110) and Huffaker (109) in population studies on strawberries in which the cyclamen mite, *Steneotarsonemus pallidus* (Banks), and its predator, *Typhlodromus reticulatus* (Oudemans), were observed on 20 pairs of field plots; in each pair one plot was kept predator-free by treatment with parathion and one had predators. In addition, on groups of greenhouse strawberry plants the predators were removed by hand. Both ways of artificial predator removal caused the cyclamen mite population in the predator-free group to increase by a similar factor, i.e., 15 to 35 times, while the mite population in the predator-infested group of plants decreased still further. Evidence was thus provided that the increase was in both cases a direct result of the removal of most of the predators, and a stimulating effect on the cyclamen mite by the chemical can be excluded. As additional confirmation of the role of *Typhlodromus* as a perimeter of cyclamen mite populations, Huffaker deliberately introduced cyclamen mites and their predators from prunings of old fields into 30 new fields, and in every case a complete biological control of the cyclamen mite was obtained. Parathion, therefore, influences the balance of arthropod population in this case in a way leading to a resurgence caused by an elimination of a natural enemy which Huffaker *et al.* had established as a limiting factor of the pest population.

Unfavourable influences on natural enemies of phytophagous species other than the pest to be controlled.—Spray applications are sometimes followed by serious outbreaks not of the pest against which they were used but of other insects and mites, which prior to the treatment were so rare in the ecosystem as not to be economically important. Such resurgences are sometimes criticized as entomological blunders and described as "man-made" pest outbreaks. The examination of "man-made" resurgences revealed in many cases a negative correlation between the population density of resurging phytophagous species and their natural enemies.

A good example was furnished by Massee & Steer (139) and Massee (138) who has made a life-long study of British fruit pests and the effect of sprays

on insect populations of orchards. They report that the red spider mite, *M. ulmi*, occurred in small numbers in many commercial apple orchards in southeast England but until 1922 not in economically significant numbers. Its population was first seen to increase in commercial orchards in 1923 after dormant sprays with tar oil had been introduced. While not toxic to the wintering eggs of the mite, tar washes had a lethal effect upon predators including the anthocorid bugs, the ladybird beetles, and predacious mites. This destruction largely accounted for the rapid build-up of mite population in the spring after their emergence from the wintering eggs, and Massee recorded the continued upward trend of mite populations in commercial orchards during the years from 1930 to 1940.

Perhaps one of the first striking cases of this sort was that in which the classical example of biological control, i.e., the cottony-cushion scale and its predator, the vedalia, *Rodolia cardinalis* (Mulsant), was upset by the use of DDT for other citrus pests in central California [DeBach (48)]. In 1946 and 1947 many thousands of acres of citrus developed damaging populations of cottony-cushion scale following DDT applications for citricola scale and other pests. Complete defoliation and crop loss occurred in some cases, and individual trees were even completely killed. This was all attributable to the elimination of one natural enemy, the vedalia, which has been recognized for years as constituting the major factor controlling the cottony-cushion scale in California. In 1948 by restriction of the use of DDT in groves obviously infested with the cottony-cushion scale, and through the appropriate timing of sprays, the resurgence of cottony-cushion scale was alleviated.

Space does not allow me to quote other recorded instances of a negative correlation between the population density of a "man-made" pest outbreak and their natural enemies after insecticide applications, but it is important to keep in mind that this undesirable effect on the natural enemies of phytophagous insects and mites is also produced by fungicides.

The effect of fungicides and insecticides on the populations of predacious thrips, *Haplothrips faurei* Hood, which is one of the most important biotic factors in the natural control of insect pests and mites in Nova Scotia orchards, was the subject of an investigation by Lord (134) and MacPhee (135). The effect of the chemicals investigated on the thrips population could be divided into three categories: (a) population elimination caused by DDT, parathion, BHC, and sulphur; (b) population reduction by dichloronaphthoquinone and phenyl mercury acetate, cryolite, nicotine sulphate, and summer oils; (c) no population reduction through ferbam, neutral copper fungicides, glyodin, arsenicals and fixed nicotine.

Lord (133, 134) in two excellent papers has demonstrated that fungicidal sprays used to control apple scab, *Venturia inaequalis* (Cooke), also have pronounced results on the balance of other arthropod populations of apple orchards in Nova Scotia. The oystershell scale had risen from a minor to a major problem, and this rise coincided with the use of mild sulphur fungicides, particularly flotation sulphur, during the past 15 years. Lord has shown that the oystershell scale has two important natural enemies, *Aphytis mytil-*

aspidis (LeBaron), and the predacious mite, *Hemisarcoptes malus* (Shimer). The mild sulphurs, such as flotation sulphur, have little toxicity to the oystershell scale, but neither of its natural enemies are able to survive when these materials are used. As *Hemisarcoptes* and *Aphytis* are the natural perimeters of the populations of the oystershell scale in Nova Scotia, the continued use of mild sulphurs often leads to an outbreak of the scale. Although lime sulphur also affects the natural enemies, this chemical is much more toxic to the scale, so that the latter does not build up as rapidly as after mild sulphur applications. Bordeaux mixture and liquid fermate have little effect on the oystershell scale and are also harmless to its enemies; where they are used, the natural enemies bring even heavy outbreaks of the scale easily under control in one or two years.

There is agreement on the detrimental effect of sulphur on the predacious typhlodromid mites which so often keep red spider mites under control. It is for this reason that sulphur causes resurgences of these tetranychid mites [Cutright (41); Flanders (65); Garman & Townsend (76); Lord (133, 134); Clancy & Pollard (30)].

Of the organic fungicides, the carbamates have a detrimental effect on the predacious typhlodromid mites as has been shown by Lord (133, 134) and by Mathys (140), who found that a heavy red spider infestation followed the treatment with carbamates, while neutral copper salts showed about one-third of the infestation found on the carbamate-treated leaves. Carbamates were found destructive to the typhlodromid mites, and trees sprayed with carbamate showed 14 times the red spider infestation of trees sprayed with neutral copper fungicides.

Enormous red spider outbreaks have been triggered off by parathion sprays in many countries. To elucidate the cause, Mathys (140) compared the effect of Diazinon, parathion, chlorobenzilate, demeton, and carbamates on *M. ulmi* on grapevines and on its principal predators of the genus *Typhlodromus*. Parathion and demeton effected the highest immediate mortality on the red spiders; Diazinon and chlorobenzilate spared a number of beneficials so that their population density remained near to that found on unsprayed trees, and no resurgence of the red spider occurred. Parathion and demeton had wiped out the predacious mite *Typhlodromus* to such an extent that there was hardly a re-establishment of this species later in the year. This resulted in a resurgence of the red spider population after application of these two pesticides.

The lethal effect on arthropods produced by dust materials, often described as "inert" on the insecticide manufacturer's label, and on natural enemies of pests has been examined by Bartlett (8). Twenty-seven "inert" dusts were examined in their effect against an aphelinid, *Aphytis chrysomphali* (Mercet), and an encyrtid, *Metaphycus luteolus* (Timberlake). Materials of mineral origin exerted a rapid killing against both species of parasites tested at a humidity of 50 per cent, whereby the thin-cuticled *Aphytis* was found much more susceptible to dry powders than *Metaphycus*. Dusts

of botanical origin were much less lethal despite observed natural adherence to the arthropod. Bartlett's work has gone a long way to explain why insecticidal dusts diluted with "inert" powders of mineral origin can be so disastrous in continental climates and lead to a resurgence of pest outbreaks. But the generality of his findings could be better assessed, if corresponding data were available for the higher humidities which occur in many temperate climates and in the tropical rain-forest.

Serious resurgences of the population of the soft scale, *Coccus hesperidum* Linnaeus, after the application of parathion were studied by Bartlett & Ewart (13). A comparison of soft scale infestation in parathion-treated citrus groves in California with untreated groves showed that parathion proved lethal to adults of *Metaphycus luteolus* for periods varying from six weeks to three months or more, depending on the dosage used. A re-establishment of the parasite in the groves once the parathion residues had dissipated resulted in rapid biological control of the soft scale. This was successfully repeated in greenhouse experiments.

Increases in the population of the frosted scale, *Eulecanium prunosum* (Coquillett), were traced by Bartlett & Ortega (14) to be a result of the deleterious action of DDT residues applied for codling moth control on the encyrtid parasite, *Metaphycus californicus* (Howard), which is an important perimeter of the frosted scale population and attacks all stages of its host.

In widely different areas resurgences of the woolly apple aphid have been observed after DDT spraying [Missiroli (149); Massee (138); Yothers (215); Baker & Porter (5)]. A negative correlation between woolly apple aphid infestation and percentage of parasitism by *Aphelinus mali* (Haldeman) in apple orchards of the Pacific Northwest after DDT sprays against the codling moth, was established by Newcomer, Dean & Carlson (151) and Newcomer & Dean (150) and compared with the correlation between aphid infestation and parasitism after lead arsenate and cryolite sprays. On trees not sprayed with DDT, the parasitism ranged from 43 to 83 per cent, and only in two out of ten orchards was the aphid infestation higher than 10 per cent of the trees. On DDT-sprayed trees, parasitism was below 10 per cent, the aphid infestation of sprayed orchards ranged from 19 to 50 per cent, and the aphid colonies were much larger. This was confirmed by Garman (74) who found resurgences of woolly apple aphid populations after all chlorinated hydrocarbon treatments, coupled with a reduction in abundance of *A. mali*.

Important resurgences of phytophagous insects in the tropics have often been observed; for example, changes in the pest population of cotton after spraying with DDT were recorded by Joyce in the Sudan (118). A DDT emulsion was applied by airplane spraying in the Gash area to control the cotton thrips, *Hemiothrips fumipennis* (Bagnall & Cameron) and *H. sudanensis* (Bagnall & Cameron), the cotton jassid, *Empoasca lybica* (deBerg.), and the cotton fleabeetle, *Podagrica puncticollis* (Weise). These treatments were highly successful against the pests but resulted in an increase of the bollworm. The mean number of living larvae of the Sudan bollworm, *Diparopsis*

watersi (Rothschild), per plant in sprayed plots was increased over unsprayed plots by approximately 70 per cent and those of the spiny bollworm, *Earias insulana* (Boisduval), by approximately 40 per cent. The DDT spraying produced 30 per cent increase in the mean number of fruiting points of the cotton plant damaged or shed by bollworm. The mean number of mature green bolls per plant damaged by bollworm was 30 per cent greater on sprayed than on unsprayed crops. Despite the good control of the jassid and the thrips, and although the DDT-sprayed cotton had per plant many more sympodic branches, more fruiting nodes, and more fruiting bolls, there was no difference between the yield of the area sprayed once and that left unsprayed. However, the area sprayed twice produced less cotton than the unsprayed one.

To prevent mass outbreaks of "man-made" pests, it has become customary to combine a further pesticide with the one causing the resurgence of the "man-made" pest. In the past such combinations of pesticides were often chosen without regard to their influence on the natural enemies of the phytophagous arthropods present, so that while they combat the resurgence of "man-made" pest species, they also frequently decrease their natural enemies and hence are likely to accelerate the appearance of long-term effects on the balance of arthropod population which will be reviewed presently. Therefore, in any ecosystem in which natural enemies of phytophagous species are present, such combination of pesticides must be regarded as an interim solution only.

Summing up, there is a great deal of evidence that many pesticides much reduce the natural enemies of the pests or of potential pests, and there is proof (13, 110) that the elimination of the natural enemies is, at least in some cases, the sole cause of the resurgence of pests.

The influence of a control of entomogenous fungi by pesticides on the balance of arthropod populations.—For the sake of completeness, mention is made of the effect of pesticides on yet another natural control factor which has a bearing on the balance of arthropod populations, namely the destruction of entomogenous fungi by fungicides (83, 84, 85, 203, 211), but shortage of space precludes a further review of this aspect of the problem.

REDUCTION OF NATURAL ENEMIES (LONG-TERM EFFECTS)

While the phenomena reviewed in the previous chapter were sudden but more or less transient, we shall now turn to influences of pesticides on the balance of arthropod population which are slow and less obvious initially but may be much more permanent. These result from the cumulative effects of reductions of the biotic resistance over longer periods in areas where regular pest control spraying programmes are carried out.

The cumulative effect of nonselective pesticides applied over a number of years, is a fauna poorer in predators and parasites of low host density dependent species, while the high density dependent species reoccur quickly and follow the resurgences often in large but strongly fluctuating numbers [Collyer (35 to 38)]. In such an ecosystem with its reduced biotic resistance

and symptoms of unbalance, resurgences of pest outbreaks after spray treatment become chronic. They occur regularly, even under less favourable climatic conditions, and "man-made" pests assume outbreak conditions. These sequels have in the past often induced the grower to increase the frequency of pesticide applications in the hope of maintaining satisfactory control; this was done even if no increase in the LD_{50} for the pest species necessitated larger dosages through a segregation of resistant strains. If the LD_{50} rose, the frequency and dosage had to be further increased.

Some research workers have, therefore, studied the effect of pesticides where they have been used regularly over an extended period of time. As early as 1929 Clark & Friend (31), working in Texas with California red scale, made monthly applications of lime sulphur solution to citrus trees and produced an extraordinary increase of red scale over that on unsprayed trees.

By monthly spraying of wettable DDT to citrus groves in California, DeBach & Bartlett (51) caused a severe disruption of the balance between the California red scale, *Aonidiella aurantii*, and its predator, *Aphytis chrysomphali*. The dosage rate was insufficient to inhibit the increase of the scale population, but it was extremely toxic to the parasites and predators. The result was that parasite activity was suppressed for 11 months, and in the course of this period the average red scale population density increase in one of the two groves so treated was 140 times and 36 times in the other. To check whether reduced parasite activity was the cause, the parasite activity was suppressed on further plots by other means, namely by giving free access to Argentine ants which guard the scales and fight the parasites off them. This led to a red scale population density 20 and 18 times higher than on trees where the ants had been controlled by 18 prior monthly applications of chlordane to the soil.

In a study of outbreaks of cottony-cushion scale, DeBach & Bartlett (51) made observations over a two-year period subsequent to DDT application. They noted that in groves where DDT treatment against citrus thrips and citricola scale had been applied prior to July, and the spray residue had weathered sufficiently before fall, the predacious vedalia re-entered and soon effected a biological control of the cottony-cushion scale. Where the groves were treated in winter or early spring and again after July, the DDT residue protected the scale from vedalia attacks throughout the entire season. Where vedalia populations built up, they controlled the cottony-cushion scale infestation and then migrated en masse to citrus trees bearing still toxic DDT residues; there the predators were killed.

From these experiments, DeBach & Bartlett (51) deduced that a grove surrounded by DDT-sprayed orchards might be materially retarded in a clean up of a scale infestation by the natural enemies as a result of the "trap" effect of DDT-treated areas on migrant adult predators or parasites. An attempt was made to produce this "trap" effect experimentally in two groves where cottony-cushion scales had built up to serious proportions as a result of previous DDT treatment (51). At the time of the experiment the

residue had weathered sufficiently so that vernalia were expected soon to recolonize the area from outside. In each orchard a number of trees were selected as test plots, and two rows of trees surrounding the plots were treated with a single application of wettable DDT. Plots of untreated trees surrounded by untreated trees 20 rows distant served as check plots. The vernalia increase in the untreated area surrounded by DDT-treated trees lagged behind and produced a less thorough reduction of the cottony-cushion scale in comparison with the check plots so that a definite "trap" action was demonstrated on the untreated area by the neighbouring DDT-treated trees. Similar "trap" effects occur on unsprayed areas of various sizes down to unsprayed parts of a tree. Michelbacher, Smith & Swanson (146) found that resurgences of the walnut aphid were caused by the greater toxicity of DDT to predators of the walnut aphid than to the aphid itself; from unsprayed islands on trees or unsprayed trees, a large number of aphids moved into the sprayed areas of the same tree or adjacent trees and built up a destructive population before the predators, which were severely reduced by DDT, could re-establish themselves.

The "trap" effect produced by residues of nonselective insecticides can lead to a serious decimation of the population of natural enemies in areas where a large percentage of the crops harbouring these beneficials are so sprayed and the spray residue is more toxic to the natural enemies than to the individuals of the pest arriving from adjacent unsprayed crops. Where such differential in toxicity exists, and natural enemies constitute a perimeter of a pest or a potential pest, the use of nonselective pesticides in large monocultures should be regarded as an interim measure until selective insecticides are available. On the other hand, if the pest is as mobile as the natural enemy, a "trap" effect of the latter does not arise, and if the pest is more mobile than the natural enemy, the pest population on untreated plots is lower than on untreated plots surrounded by untreated fields.

Many economic entomologists have observed that an increasing dosage of nonselective insecticides becomes necessary when insecticide applications are repeated over a number of years, and there are cases in which an increase in the medium lethal dose for pests consequent upon repeated spray application has been established. This phenomenon is, of course, the beginning of the development of resistance of arthropods to pesticides and has been studied in that connection. But before a higher degree of tolerance to pesticides is acquired, it may be noted here as a long-term effect of pesticides on balance of arthropod population and illustrated by one example: Ivy & Scales (113) compared the LD_{50} and LD_{90} for a number of cotton pests over six years. The toxicity of toxaphene to the cotton leafworm, *Alabama argillacea* (Hübner), rose in pounds per acre calculated as LD_{50} from 0.17 to 1.62, while the LD_{90} rose from .60 to 5.85, a nearly tenfold increase. Equally, for *Tetranychus tumidus* Banks the LD_{50} rose from .014 for untreated populations to .167 and the LD_{90} from .036 to .462. No increase in the minimum lethal dose was encountered over the same period with the cotton aphid or boll weevil.

Long-term effects of pesticides on the balance of arthropod populations are more obvious on perennial crops but occur also on annual crops where the sprayed area in a territory amounts to a high percentage of that crop and the pests and their natural enemies are oligophagous. When the pests are polyphagous and the sprayed area is only a small percentage of the crop, only short-term effects of the pesticide are observed.

Reduction of the natural enemies by treatments leading to a permanent lowering of the environmental resistance, is a condition which needs attention; the rapidity of the resurgences caused by lowered environmental resistance is understood when the astronomical figures of the biotic potential uninhibited by the environmental resistance are considered. This was illustrated about 100 years ago by Huxley (1858) who calculated that a single parthenogenetic female aphid could give rise in a year's time to a progeny whose total body weight would be equal to that represented by the inhabitants of the Chinese empire of his time. This oversimplified but alarming calculation was examined by Herrick (94) who found the reproductive capacity of the cabbage aphid in a good year to lead to 12 generations between March 31 and August 15. A single stem mother produced an average of 41 young aphids; on this basis the progeny, if they all lived, would number over half a trillion. As the average weight of four aphids is 1.4 mg., the total number of aphids would weigh one and a half billion pounds, or 250 million tons. These theoretical figures have obviously to be used with reservation, but they serve to understand the phenomenon of the resurgences caused by a lowering of biotic resistance and underline the necessity to preserve forces which help to prevent such grotesque multiplication in nature.

FAVOURABLE INFLUENCES OF PESTICIDES ON PHYTOPHAGOUS ARTHROPODS

Stimulation of natality.—In addition to the reduction of the environmental resistance to phytophagous arthropods reviewed briefly in the previous section, there is evidence of favourable effect of some pesticides on the biotic potential of arthropods.

The now notorious increases in the population of red mite associated with DDT treatment were first noted in 1944 [Steiner, Summerland & Fahey (188)]. Some authors at first thought them to be solely attributable to an elimination of the natural enemies of the red spider mites, while others posed the question whether this was caused by a destruction of the natural enemies or by a change in the physiology of the mites or their host or both (57). Huffaker & Spitzer (111) working with the two-spotted mite on pears came to the conclusion that DDT treatments produced an increase of mite population above that occurring on check trees which was not explainable by a reduction of the natural enemies.

Further light was shed on this in Professor Kuenen's laboratory at Leiden by an interesting investigation on the population dynamics of the fruit tree red spider in Holland [Hueck *et al.* (107); Hueck (106)]. Apple trees with a moderate infestation of red spider mites were sprayed with parathion which

killed all mites which were not in the egg stage, and all predators. After the application of parathion, a number of trees were sprayed with DDT suspension, and for the following months the numbers of all stages of red spiders were ascertained every second day. An examination of the resulting data led Hueck *et al.* to the conclusion that egg production had been substantially larger on DDT-sprayed trees than on the check trees. In laboratory experiments in which leaf discs were dusted with 5 per cent DDT, there was again a significant increase of the egg production under the influence of DDT dust. The Dutch investigators' experiment was not designed to explore whether the stimulating factor acted as a direct influence of the spray chemical or via the plant, or was translocated in the plant. The phenomenon requires further confirmation, and it is desirable that this interesting short-term effect on the balance of arthropod populations is studied also for representatives of other groups of arthropods so that its generality can be assessed. To this end Kuenen (125) has made further progress in demonstrating a similar phenomenon for *Calandra granaria* (Linnaeus) where he finds that low DDT concentrations stimulate egg production by 20 per cent, while DeBach *et al.* (54) were unable to find this phenomenon after DDT treatment of citrus scales.

Indirect influence of pesticides on the balance of arthropod population through the host plant.—As soil fertility and adequate nutrition greatly influence the condition of plants and thereby the number of phytophagous insects which can maintain themselves on them [Holloway, Henderson & McBurnie (102); Kuenen (123); Osburn & Mathis (157); Rodriguez & Neiswander (175); Garman & Kennedy (75); Haseman (92)], nutrient sprays alone or in combination with pesticides applied to deficient plants may form an important indirect influence on the population of arthropods and increase the number of phytophagous species and after the normal time lag, their predators and parasites, if they do not have long-term residual effects. For example, Thompson (196, 197) showed in Florida that the use of zinc and copper resulted in trees with fewer nutritional deficiencies, and the more vigorous growing trees were found to be more satisfactory hosts for the purple scale, *Lepidosaphes beckii* (Newman), and the Florida red scale, *Chrysomphalus ficus* Ashmead. Equally, where magnesium deficiency had been corrected and trees had a higher percentage of green leaves, purple scales were more abundant than on trees with a higher percentage of bronze leaves where magnesium deficiency still existed.

Plants are also influenced by DDT, as Chapman & Allen (28) and others have shown. In turn, red spider mites are influenced by the condition of plants [Kuenen (124)] so an indirect influence of the spray chemical via the plant on this mite cannot be ruled out. The resistance of plants to attack by the citrus red mite, *Metatetranychus citri* [Ebeling (61)], was studied by Fleschner (66) who found that the degree of host resistance to citrus red mite attack ranges from almost complete immunity to great susceptibility. In an interesting experiment, citrus terminals which were mite-free were treated

with DDT, zinc, and field dust. After treatment they were artificially infested with mature female citrus red mites; during three generations of citrus mites fewer eggs were laid on the check terminals than on either the DDT-treated or the zinc-treated terminals.

Fleschner also obtained an indication that the factor which leads to the increase in the number of mite eggs is translocated 46 days after treatment but not before, and only in the case of DDT. He interpreted these results as a lowering of the host plant resistance through the treatment with DDT, zinc, or field dust, but they could also be interpreted as increased biotic potential of the phytophagous arthropod. A verification by further experiments will have to be awaited until the conclusion of an indirect stimulation through the plant or a translocation of such effect can be considered established.

THE INFLUENCE OF PESTICIDES ON THE BALANCE OF ARTHROPOD POPULATIONS THROUGH REMOVAL OF COMPETITION OF PHYTOPHAGOUS SPECIES

In contrast to the fruitful investigations made into interspecies competition between pests of stored products, there is little information on the extent to which phytophagous insects and mites compete for their food supply and few references to the reaction of arthropod populations when competition is removed by pesticides eliminating the population of one of the competing species. The removal of competition by the jassid *Empoasca lybica* cotton by DDT spraying would, according to Joyce (117), "inevitably result in an increased whitefly population, *Bemisia tabaci* (Gennadius), even if parasites were not involved" because competition between the jassid and the whitefly is normally won by the jassid, which damages the cotton leaf seriously; but Joyce's statement is unfortunately not supported by published experimental data.

The competition of the European red mite, *M. ulmi*, in New York apple orchards might be an important factor in the suppression and late build up of the two-spotted mite, *Tetranychus telarius* [Ebeling (60)], because the latter appeared earlier and in much larger numbers than the European red mite and was virtually eliminated for the season by preblossom sprays against the first brood [Lienk & Chapman (132)].

The evidence available is too scanty to assess what influence the removal of competition through pesticides has on the balance of arthropod populations and whether this factor may cause or contribute to a resurgence of potential pests after a chemical control of the phytophagous species which dominate the habitat.

SEGREGATION OF RESISTANT STRAINS

One of the most important effects of pesticides on the balance of arthropod populations is the acquired resistance to insecticides, and there has been more research and general discussion of this long-term effect than of any other. Out of 5,000 species of economically important insects, 23, that is, $\frac{1}{2}$

per cent, have formed resistant strains. Since this phenomenon has recently been excellently reviewed by Metcalf (142) and by Wigglesworth (207b), we have to concern ourselves here only with the role which alterations in the balance of arthropod population have on the development of resistance. Resistance may result either from the selection by the pesticide of the most resistant individuals in a population, or by interaction of pesticide and arthropod to produce a resistant adaptation; most investigators accept the first alternative and concur that resistant strains originate from genotypic variants. Resistant survivors of pesticide treatment must, therefore, be the target of any attempt to prevent this undesirable phenomenon. Mixtures of pesticides with two independent, uncorrelated modes of action have been suggested [Hewlett & Plackett (96)] as a possible method. This is analogous to the mixture of antibiotics used to prevent resistance of bacteria from developing too rapidly and also bears comparison with the alternation of weedkillers practised in order to prevent the substitution of susceptible species of weeds by others which do not succumb to the weedkiller (1, 169). But the analogy with antibiotics and the rotation of weedkillers is only superficial because there is in these cases no parallel for the repercussion of the pesticide on the natural enemies of the uneconomic organism.

Using the knowledge of cross tolerances of resistant insect strains as a guide, experiments are in progress to prevent the development of resistance by mixtures of pesticides with independent, uncorrelated modes of action and also by the employment of alternative treatments with such pesticides (43). This method has in our opinion its place in habitats where, at the time of application, no natural enemies of the pest are present to form a perimeter of the population of the pest. It may also possibly be used on annual crops where the area to be sprayed is only a small percentage of the total acreage under such crop and where the pest is polyphagous, so that the treated pest population form only a small percentage of total population present in the district. Where natural enemies, however, keep the phytophagous arthropods in check for part of the time on perennial crops or on annual crops, and where a large percentage of the acreage is sprayed and the majority of the population of the pest in the district is thus involved, the application of nonselective mixtures of pesticides will effect a decrease of the biotic resistance which might produce other sequels than the segregation of resistant strains.

A better way to prevent the selection of resistant strains through shifting the balance between the pest population and its natural enemies in favour of the latter, will be discussed in a later section of this review.

THE PREVENTION OF UNFAVOURABLE EFFECTS BY PESTICIDES ON THE BALANCE OF ARTHROPOD POPULATIONS

To avoid unfavourable effects by pesticides on the balance of arthropod populations, research work has been carried out along two different paths:
(a) to apply nonselective chemicals in such a way and at such time or dosage

level that effects on arthropods other than the pest species in the ecosystem are minimized, and (b) to develop selective pesticides which are more or less specific against the injurious phytophagous arthropods and do not kill their natural enemies.

Reduction of adverse effects on natural enemies by timing of the application.—In some cases it has proved possible to minimize the damage to the natural enemies by timing of pesticidal sprays before the majority of the predators and parasites become active, but the scope of this method is limited. Shortage of space permits only a few examples.

Based on a study of the seasonal host parasite relationship of the frosted scale on English walnuts in California, Bartlett & Ortega (14) showed that ordinarily DDT sprays against codling moth are applied during the period when they are least detrimental to the parasite activity. Heavy dosages and double treatment dangerously extend the period of the harmful DDT residue, eliminate the predominantly effective natural enemy, *Metaphycus californicus*, and then serious outbreaks of the frosted scale occur. Carefully timed applications of DDT as dust or spray to control diabrotica beetles and leafhoppers of melon were found not to interfere with the balance between the melon aphid, *Aphis gossypii* (Glover), and its predators, the principal of which was *Hippodamia convergens* (Guerin) (143).

On similar lines, Massee (138) in England and Geier (77) in Switzerland have recommended a modified spraying programme which reduces or avoids resurgence of outbreaks by fruit tree red spider, *M. ulmi*, through timing the DDT treatment of apple trees at the green cluster stage of bud development.

A very early forest dusting against the nun moth, *Lymantria monacha* (Linnaeus), in Germany killed only half of the caterpillar population, but as the important tachinid parasite, *Phorocera silvestris* (Robineau-Desvoidy), had not hatched before the treatment and were as pupae in the soil, it produced so high a percentage parasitism as to eliminate the pest outbreak (70).

Reduction of adverse effects on natural enemies by alteration of the application method.—The method of application can make a difference in the influence of chemicals on the balance of arthropod populations. Severe whitefly (*Bemisia tabaci*) outbreaks on cotton became a regular sequel to DDT spraying against the cotton jassid, *Empoasca fabae* (Harris), in the Sudan when high volume application was replaced by low volume spraying. Norman (155) has shown that the increase of whitefly populations after high volume spraying (100 gallons containing the same amount of DDT, i.e., 1 lb. as emulsion) was very much smaller than after low volume spraying and exceeded the whitefly peak on unsprayed crops only insignificantly, although the whitefly population remained on the high volume DDT-sprayed crops at this level for five weeks, while on the unsprayed crop it declined steadily during the same period.

If a certain proportion of the plant population only can be treated at a given time, the natural enemies may be relatively unaffected and can continue their work as perimeters of the population of phytophagous species by

quickly recolonizing the strips which have been sprayed and where the natural enemies were decimated. DeBach (54) has demonstrated that a strip treatment for control of the purple scale is compatible with the conservation of natural enemies. In an orange grove where red scale was not completely under biological control by *Aphytis chrysomphali* each third pair of rows of trees was treated at six monthly intervals, beginning with the first pair; then six months later the second pair of rows and six months after that the third pair were sprayed with an oil spray. This cycle was repeated, and a rapid dispersal of natural enemies took place from untreated trees back to the treated trees soon after treatment. A good scale control resulted, and neither the California red scale nor other pests became plentiful.

Minimization of adverse effects on the balance of arthropod population by a choice of the pesticide least toxic to the natural enemies.—Several investigators have made great progress with this approach, pioneered by Pickett and his collaborators, Lord, Patterson, MacPhee, and Sanford (133 to 136, 160 to 164) in Nova Scotia, and by Bartlett (9) and DeBach *et al.* (54) in California.

By finding out which species of the natural enemies of the pests is an effective factor in its biotic resistance, and by adapting ecological methods to chemical control studies to clarify the direct and indirect influence of the spray programme on the fauna, a number of spray schedules were worked out and tried over a number of years. Thus resurgences of the oystershell scale, the European red mite, and the eye-spotted bud moth were avoided by substituting sulphur applied against the apple scab, *Venturia inaequalis*, with copper and ferbam, glyodin and captan. Glyodin and captan have no detrimental effect on the predacious typhlodromid mites [Lord (133, 134); MacPhee (135); Garman (74); Clancy (29); and lately Collyer & Kirby (39)]. The last authors compared dispersible sulphur, lime sulphur, glyodin, and captan in their effect on the European red mite and its typhlodromid predators and found that the typhlodromid population increased on the plots sprayed with organic fungicides and remained high throughout the season. On the sulphur fungicide plots, on the contrary, the typhlodromids were reduced each spring. Typhlodromids in England play a decisive role only if the rate of the pest to that of the predators is low, and such a low ratio was only retained in the organic fungicide plots, while the ratio on the lime sulphur plots was always unfavourable and rose in mid-summer to high values; on the plots sprayed with dispersible sulphur they remained in an intermediate position.

The success of Pickett's approach was confirmed by Ross (177b) and Le Roux (131) who in Quebec compared two different spray programmes in their effect on the densities of natural enemies which indirectly affect the densities of the injurious phytophagous arthropods on 1,250 tree plots over four years. The first, a "modified spray programme," was a programme where corrective sprays only were used to control insect and mite pests, and preventative fungicides were used for the control of apple scab; only spray chemicals known to be relatively innocuous to natural enemies of apple pests

were chosen and used in the first programme. The second, a "commercial spray programme," was based largely on preventative spray measures for both insects and scab, as used by the average fruit grower guided by the annual spray calendar.

The "modified spray programme" consisted of an average of 8.3 fungicidal and 2.8 insecticidal sprays at an average spray chemical cost of 0.63 cents per tree and resulted in a build-up of beneficial forms and a reduction of the pests. The average yield per tree of insect-free apples was six bushels, and the control was very good with less than 25 per cent of the crop damaged.

In the "commercial spray programme" an average of 12.2 fungicidal and 5.4 insecticidal sprays were applied at an average cost of \$1.14 per tree (spray chemicals only). This resulted in the practical elimination of all natural enemies with the maintenance of a corresponding high density of injurious phytophagous arthropods. The average yield per tree of insect-free apples was $4\frac{1}{2}$ bushels per tree, and from 25 per cent to 35 per cent of the crop was damaged.

The natural re-establishment of predators and parasites after pesticide treatment has been investigated by DeBach (49) and Collyer (36) and the comparative toxicity of persistent residues to natural enemies by Bartlett (11), but in addition some workers have attempted to correct the unfavourable effects of nonselective pesticides on the natural enemies by releases of natural enemies after treatment (25, 73). The effect of toxic residues of DDT, BHC, and parathion on *Cryptolaemus montrouzieri* (Mulsant) was studied by Sýsoev (194). DDT and BHC killed adult ladybirds and larvae released on the treated plants up to 30 days after treatment, and BHC had in addition a repellent effect for at least two months. After parathion treatment the coccinellids could be released 20 days after treatment without loss. After sulphur dusting all the adults and most of the larvae died if they were released up to 20 days after treatment in the greenhouse, but in the open the beetle left the plants when released 15 days after sulphur treatment. However, they became established and devoured the mealybugs, *Planococcus citri* (Risso), when released 30 days after sulphur dusting.

Shift of the balance of arthropod population in favour of the natural enemies by selective pesticides.—Although selective toxicity has dominated chemotherapy in medicine since Ehrlich's work in the first decade of our century, in entomology the conception of selective toxicity with regard to natural enemies seems to have been formulated only in 1944, when a selective insecticide was defined as a chemical which kills the "uneconomic" arthropod species and spares the economic species, namely the pest's natural enemies (168). As first example, we used the selectivity of nicotine at short exposures which produced a high mortality of the cabbage aphid, *Brevicoryne brassicae* (Linnaeus), while the predators of this pest, namely *Coccinella septempunctata* (Linnaeus) and *Adalia bipunctata* (Linnaeus), the larvae of syrphids, *Syrphus ribesii* (Linnaeus), *S. balteatus* (Degeer), and *Lasiophthicus pyrastris* (Lin-

naeus), the larvae of cecidomyiids of the genus *Phaenobremia*, and the parasite *Diaeretus rapae* (M'Intosh) were not killed. Nicotine dust had similar effects on the melon aphid and its predators and parasites when applied under a drag sheet (201).

In 1949 the exciting discovery was made that the systemic insecticide, schradan, was toxic to a number of phytophagous arthropods such as aphids, whiteflies, scale insects, and a number of spider mites but did not kill their predators, the coccinellids, *Coccinella septempunctata*, *Adalia bipunctata*, nor syrphid larvae, nor the parasite *Diaeretus rapae* (172). Schradan was also found not to affect the larvae of *Phaenobremia* preying on aphids (170) nor the predator *Blepharidopterus angulatus* (Fallen), while his prey the greenhouse red spiders were satisfactorily controlled (171). Schradan did not kill anthorcorids, mirids, or capsids against which it was tried, and references to this extent (162) are unconfirmed. As already mentioned (see p. 412), schradan kept aphid populations low for at least 40 days, while the nonselective organo-phosphorus pesticides, parathion and para-oxon, produced a rapid resurgence of the pest population within 14 days (171).

By the use of a selective insecticide, the balance of the arthropod population can be shifted so much in favour of the natural enemies that the latter spill over into adjacent untreated pest populations and effect biological control which is triggered off by the selective chemical used on the neighbouring treated area. In experiments in which a heavily blackfly-infested sugar beet field was divided into plots of 40 ft. width and alternate plots were sprayed with schradan, an excellent control of the aphids resulted. Within three days after the complete elimination of aphids on the sprayed plots, all the predatory coccinellids had moved to the untreated plots; there the coccinellids cleared up all the aphids within the next four days, so that on both sprayed and unsprayed plots the aphid outbreak disappeared within 10 days, while outside of the experimental area the infestation continued. This is an example of influencing the balance of population by insecticides without actually spraying the whole area (170).

As schradan does not kill arthropods by contact with their cuticles (80) nor predators by ingestion of aphids killed by schradan, this type of selective action has been termed physiological selectivity (172). The selectivity of this systemic insecticide was confirmed by Geier (77) against the fruit tree red spider in Switzerland, and by Wright & Wheatley (214) in a very interesting investigation comparing the effectiveness of organo-phosphorus insecticides for the control of the cabbage aphid.

Schradan is used in Britain on a field scale against aphid outbreaks on annual crops and is accepted as regular treatment on certain permanent crops. Over 90 per cent of the British hop acreage has for the last six years been treated with schradan with very good results. The use of this selective systemic insecticide has meant a very sizable reduction in the number of pesticide treatments required to control the hop aphid, *Phorodon humuli* (Schrank), and the hop red spider mite, *Tetranychus telarius* [Jary (114)].

Good results on another permanent crop were obtained by Collingwood

(33) on gooseberries who noted virtual freedom from the red spider, *Tetranychus telarius*, for some 10 weeks after one schradan application, while two applications effected freedom for the whole season. The beneficial insects, *Stethorus punctillum* (Weise) and *Oligota flavicornis* (Erichson), were found to be in a direct relationship to the number of mites (34). This performance (33, 77, 114) seems to confound Pickett's doubts (162) in the value of selective systemic insecticides for combining chemical with biological control in a permanent type of plantation.

By contrast, the systemic insecticide, demeton, shows contact toxicity to predators (40) and parasites (2), and the same is true for metasystox; the effect of demeton on predators after ingestion of aphids killed by sucking on demeton-treated plants was also investigated. Three species of Syrphidae were regularly killed, and the susceptibility of five coccinellid larvae tested ranged from 100 per cent mortality in *Scymnus haemorrhous* (LeConte), to 3.7 for *Ceratomegilla maculata* (Degeer). Adult coccinellids were not affected except *Cycloneda sanguinea* (Linnaeus) of which 50 per cent were killed. Larvae of *Chrysopa* were not affected (2).

Equally, we found in England that metasystox kills a high percentage of predators of the bean aphid, *Aphis fabae* (Scopoli), and cabbage aphid, *Brevicoryne brassicae*, i.e., syrphids, ladybird larvae and parasites by contact as well as the aphids. Hence resurgences of bean aphid and cabbage aphid populations occurred within 14 to 20 days after metasystox application and three weeks after demeton application.

A search for a selective acaricide for strawberries was undertaken by Huffaker & Kennett (11), and recently Huffaker (109) succeeded with aramite to kill the two-spotted mite, *Tetranychus telarius*, which forms a control problem in California, without repercussions on the typhlodromid mites, who on their own are adequate to keep cyclamen mite, *Steneotarsonemus pallidus* (Banks), under control. The same compound was found to be effective against *Tetranychus telarius* and *T. atlanticus* McGregor on seed crops of alfalfa and red clover in Washington, while predator populations remained high [Klostermeyer (121)].

p-Chlorobenzyl-*p*-chlorophenyl sulphide has proved toxic to the immature stage of *Metatetranychus ulmi* and *Tetranychus telarius*. Residues on foliage exert a persistent ovicidal effect and act as stomach poisons on the nymphs even at concentrations at which they are not ovicidal. Adults are not affected, but an active infestation in the field is reduced to negligible proportions in 2 to 3 weeks because of the acaricide's persistent toxicity to immature stages, while no insecticidal effects were observed and active populations of the predator, *Blepharidopterus angulatus*, coccinellids, syrphids, anthocorids and the predacious mite, *Typhlodromus* sp. were found in sprayed orchards [Cranham, Higgons & Stevenson (40)].

Experiments to find a selective insecticide against pests of apple orchards has been conducted by Clancy (29) since 1952. In an effort to find a substitute for DDT against the codling moth but without its harmful effect on the natural enemies, ryania was first used in 1952. It gave control of the

codling moth but was harmless to predators, so that the red mite predator balance in several of the ryania plots compared favourably with those in the unsprayed plots, indicating a successful biological control (29, 163). Less favourable results on the braconid parasite, *Macrocentrus ancylivorus* Rohwer, and other parasites of the oriental fruit moth, *Grapholitha molesta*, were obtained with ryanex (206).

Another way of obtaining physiological selectivity was attempted by using a nonselective insecticide, whose particles were coated with a material which limited the contact effect to nonphytophagous beneficial insects, while stomach toxicity for phytophagous arthropods was released through mastication or digestion of the coating of the particles (173).

A much larger effort to find physiologically selective insecticides is obviously desirable. Bartlett (12) started to screen one beneficial insect per week against about 50 pesticides in order to determine the selectivity of these materials against the most important Californian parasites and predators.

Where physiological selectivity cannot be brought into play, it is sometimes possible to exploit differences in the ecology of the pest and the natural enemies to produce a selective effect; thus we were able to convert nonselective systemics, which are well translocated in the plant's sap stream, into insecticides with ecological selectivity (172). Dimefox (coded in 1951 as CR 409) was found toxic to *Brevicoryne brassicae* and killed by contact *Coccinella septempunctata*, syrphid larvae, and *Diaeretus rapae* but when applied to the roots of brassicas, gave a good mortality of the cabbage aphid without affecting the beneficial insects. In a similar way, a high mortality of *Tetranychus telarius* was obtained by a soil treatment around the hop plant with dimefox without affecting the predator, *Blepharidopterus angulatus*. We also showed that the mealybug, *Planococcus kenyae* (Le Pelley), of coffee can be killed by a soil treatment (172). Jeppson (115) and Bond (19) have demonstrated the same effect by a treatment of the bark with demeton against *Planococcus citri* and dimefox against *P. kenyae* respectively. After the application, systemic insecticides are translocated to organs of the plant where the natural enemies are not killed because they are not reached by the contact effects of the systemic insecticide. Along the same lines, the cocoa mealybug, *Pseudococcus njalensis* (Laing), was controlled by dimefox treatments applied around the stem of the cocoa tree or by trunk implantation without affecting the pollinating ceratopogonid midges nor various predators preying on the mealybugs (91).

All the available evidence shows that a plant population, such as a crop or an orchard, accumulates after infestation by phytophagous arthropods, a defence mechanism against the phytophagous insects and mites in the form of a population of natural enemies. In the majority of cases this defence mechanism can only cope with moderate populations of phytophagous arthropods and severe outbreaks can only be controlled in time by chemical methods. Even if selective pesticides are used, this defence mechanism of the crop might ultimately be upset.

Boyce (21), Nicholson (153), and Wigglesworth (207a) warned that a

reduction of a phytophagous species to a low population density by chemical control could produce an increase of its biotic potential as a consequence of the simultaneous reduction of biological control factors, whether attributable directly through the insecticide or indirectly to its being starved out. That this latter phenomenon can also occur with selective insecticides is obvious from our early experiments (168), and the question arises whether there are better ways of using selective insecticides.

In an ecosystem where natural enemies are effective, a pest outbreak occurs when the biological control of pests becomes inadequate; if selective chemical control could then be so used as to offset only the effect of factors which have upset the balance of population without so reducing the pest population that the natural enemies eliminate them completely and then disappear themselves, a new equilibrium between the pest and the natural enemies on a lower level of population density would establish itself. In all cases of pest control problems other than the control of virus vectors, the target level to which populations of the pest species should be reduced is determined by the population density of the pest species which does not produce damage to the crop, taking into consideration the population density of the pests' natural enemies present in the ecosystem and their biotic potential. The way to determine the nondamaging population density has been shown by Strickland (191) who correlated population densities of several crop pests with yields of the marketable produce. By experiments on a great number of sites in different parts of the country, correlations of considerable generality were obtained from which the maximum nondamaging pest populations per unit area can be extrapolated.

To test the hypothesis of re-establishing a balance between the pest population at a new economically insignificant level and its natural enemies by selective insecticide, we used again cabbage-aphid-infested brassica crops as a suitable ecosystem. We lowered the cabbage aphid population in a great number of plots with reduced concentrations of systemic aphicides in order to produce levels of aphid population after treatment below the population density indicated by Strickland as not doing significant damage to the crop. With 20 per cent reduction of the schradan concentration generally recommended for cabbage aphid control, we were able to reduce the cabbage aphid infestation to a density which the low level natural enemies found sufficiently high to exist on, and which they were able to keep under the population density causing damage at any time during the season. Similar reduction in dosage of demeton and metasystox were unsuccessful and led to resurgences.

Where selective insecticides have to be used early in a cabbage-aphid outbreak when the natural enemies consist largely of low density dependent factors, a considerable degree of selectivity and the use of high concentrations of selective insecticide is required, but later on in the outbreak when low and high density dependent natural enemies are present, considerable economies in the dosage of the chemical can be made. Work is now in progress to collect data on which recommendations for the reduction of the dosage can be based in accordance with the composition of the fauna of the natural enemies and

the density of the important natural enemies. In the control of aphids on brassica crops an increase of the biotic potential of the pest as a consequence of the reduction of the biological factors as a result of their being starved out was thus avoided. This method not only minimizes the number of applications and reduces the dosage of the selective pesticide but also reduces the risk of toxic residues at harvest time. It remains to be seen whether the ecosystems on which these experiments were carried out are exceptionally simple in the composition and interrelation of constituent arthropod species, or whether this method has wider applications.

Prevention of the development of resistance.—The use of selective insecticides seems also to form a practical way to prevent resistant survivors of pesticide applications from multiplying because survivors of chemical treatment are eaten by the predators and parasites whose food supply has been drastically reduced by the action of the selective pesticide; this hypothesis occurred to the writer (168) when studying the clean up of survivors after a selective chemical treatment by the natural enemies. The surviving syrphid larvae concentrated for three days following treatment on devouring the survivors of the cabbage aphid population, then left when the population density of the aphid had become too low, while *Diaeretus rapae* continued its parasitism until 100 per cent of the survivors were parasitized, and a complete clean up of all survivors had been effected. We also studied the clean up of the surviving aphids following an application of schradan for the selective control of the cabbage aphid and found it similar to that effected by the somewhat cumbersome nicotine fumigation (174).

While the hypothesis of the prevention of resistant strains by the use of selective pesticides has not been proved in clear-cut experiments, there is no case recorded where selective pesticides have been used and a resistant strain has been segregated in those cases where pesticide treatments were carried out in ecosystems where natural enemies were present.

EFFECT OF SOIL INSECTICIDES ON THE BALANCE OF SOIL ARTHROPODS

Where soil pests are to some extent controlled by natural enemies it is to be expected that phenomena similar to those already described would occur in the soil when phytophagous arthropods are controlled by soil insecticides which kill the soil pests' natural enemies along with the pest, but further phenomena relating to the balance of soil arthropods are likely because the ecosystems in the soil are inhabited by a far larger fauna of arthropods than the plants above ground; there are large numbers of microarthropods living in the soil of arable land chiefly belonging to Collembola, Oribatei, and Mesostigmata [Franz (68); Kühnelt (126)]. Soil biologists have called attention to the role of the Collembola and acari in the production of humus and fertility of the soil [Stöckli (190)].

The great success of soil insecticides in the control of soil pests and their increasing use raises the question to what extent soil insecticides affect the

microarthropods and natural enemies of soil pests. The literature has recently been reviewed by Satchell (178) and shows many gaps in our knowledge. All investigators agree that the balance of arthropod population is indeed considerably affected. Not only are many soil pests effectively controlled but the population of microarthropods is temporarily much reduced (15, 119, 167, 180, 181) by BHC, parathion, and DDT. But DDT-treated soil thereafter shows a significantly higher population of Collembola than the untreated soil, while benzene hexachloride leads to significant decrease of all groups of microarthropods.

Further information on the population changes produced by DDT were given by Sheals (181). At least five mesostigmatic mites commonly occurring in the field, preyed actively on the Collembola, in particular *Pergamanus* species. Laboratory observation showed DDT was toxic to the Mesostigmata, while Collembola were completely unaffected by DDT; in the field, significant increases of Collembola in the DDT-treated plots were associated with a significant reduction of Mesostigmata.

As the influence of the soil insecticides, BHC and DDT, are of a transient nature, von Baudissin (15) comes to the conclusion that cultivation has a much more permanent influence on the balance of microarthropod population in the soil than the soil insecticides, BHC and DDT. This opinion is not shared by Sheals (181) who is concerned that adverse long-term effects on fertility might occur as a result of general decimation of saprophagous species by BHC, and points out the risk of DDT favouring phytophagous Collembola.

Until investigations are carried out into the influence of all groups of the most effective soil insecticides on the natural enemies of the soil pests and the microarthropods it is impossible to make any general assessment.

CONCLUSION

A review of the influence of pesticides on the balance of arthropod population would be incomplete without a few deductions on the application of the reviewed information to practical problems of control of agricultural pests.

(a) To assess a pesticide treatment the composition of arthropod fauna and the adjustment of the population density of the economically important arthropod species over a number of years and after several applications should be studied. Such studies are necessary in order to eliminate pesticides or application conditions which lead to resurgences of pest populations, which necessitate increases in dosage, and which might produce a segregation of resistant strains.

(b) There are many cases of pest occurrence where the pest has no natural enemies in the habitat when chemical control is applied. Provided that there are no other potential pests present which have natural enemies as perimeters of their population density, there can be no objection to the use of nonselective pesticides; where these have a favourable effect on the biotic potential of any of the phytophagous species, the mixture of a second toxi-

cant with an independent, uncorrelated mode of action to combat the stimulated species is practical.

(c) Where natural enemies are present which on their own for part of the time or supplemented by chemical treatment can control a pest or a potential pest, then selective insecticides or the use of nonselective insecticides in a way which avoids unfavourable influence on the natural enemies and stimulation of the phytophagous species, succeed in keeping crop protection costs at a minimum and avoid the sequels mentioned in (a) above.

The satisfactory performance of selective pesticides justifies a far wider study of selective toxicity differentiating between economic and uneconomic arthropods. Such work has been commenced on the lines of ascertaining the toxicity of existing pesticides not only to pests but also to their natural enemies and of searching for new selective compounds and selective formulations.

(d) The generally used method of combating resurgences of phytophagous arthropods, previously biologically controlled, by adding a second non-selective pesticide where natural enemies are present further decreases the biotic resistance of the habitat and can, therefore, safely be practised only in cases where the sprayed area constitutes a low percentage of the crop in the district, or where the pest to be controlled is polyphagous and there is thus a large population of the same pest species on food plants outside the sprayed area.

For the prevention of the development of resistant strains in ecosystems where the natural enemies are present, the use of selective insecticides seems the most promising approach, while the alternatively suggested method, namely the employment of a mixture of two nonselective pesticides of independent uncorrelated action or alternate treatments with such materials even if successful, would again make a decrease in biotic resistance of the treated habitats often unavoidable.

(e) The application of fungicides by plant pathologists sometimes interferes with the natural enemies of phytophagous arthropods so that the entomologists have to apply further chemical treatment, and the same is to a certain extent true for the application of plant nutrients. It might, therefore, be well to consider what steps should be taken to co-ordinate the efforts of the various specialists recommending chemical treatments for one crop so that the effects produced do not clash and offset each other.

(f) The relative importance of natural enemies has been elucidated for a number of important pests, but a knowledge of the natural enemies of major effectiveness as a perimeter of pest populations for a much larger number of pests will be required in any general attempt to devise a chemical control in supplementation to biological control. This is obviously a gigantic task; it will also take time to develop selective pesticides or spray programmes compatible with biological control for pests in all those habitats where effective natural enemies are at work or are available. On pest problems for which selective pesticides are not yet available, the use of nonselec-

tive pesticides will, in the meantime, have to go on, but for habitats where natural enemies are present, this must be regarded as an interim solution which is becoming steadily more costly and may have its dangers.

(g) A review of the effect of pesticides on the balance of arthropod populations shows that a plant population such as an orchard or a growing crop can be considered as a biological system designed to produce a maximum of food for humans, which very frequently has as a defence mechanism against phytophagous insects and mites, a population of their natural enemies. Like the defence system against foreign bodies and microbiotic infections in vertebrates, this defence mechanism can only cope with moderate infestations. Only pesticide applications have so far succeeded to correct heavy infestations by phytophagous pests, but these proved more successful in the long run if they were so selected as to eliminate or reduce the offending pest population only, and thus supplement the defence mechanism, not destroy it.

As A. W. A. Brown has put it so concisely: "In crop protection the chemical weapon must be used as a stiletto, not a scythe."

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